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Structure And Function Of Glands Producing Airborne Semiochemicals In Four Species Of Lepidoptera

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STRUCTURE AND FUNCTION OF GLANDS PRODUCING AIRBORNE SEMIOCHEMICALS IN FOUR SPECIES OF LEPIDOPTERA

by

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ABSTRACT

The structure of the sex pheromone gland was studied in females of three species of moths, the spruce budworm, Choristoneura fumiferana (Tortricidae), the whitemarked tussock moth, Orgyia leucostigma (Lymantriidae), and the cabbage looper, Trichoplusia ni (Noctuidae). In all three species the gland is everted by several muscles which are identified and described.

The ultrastructure of sex pheromone gland cells, before and during release of the sex pheromone, was compared. Gland cells exhibit similarities during development and maturation indicating extensive lipid metabolism. Adult gland cells in each of the three species contain smooth endoplasmic reticulum and well-developed microvilli each of which contains a tubule of smooth endoplasmic reticulum. In the spruce budworm, lipid spheres are located near the bases of cells and do not vary in number or size. In the cabbage looper lipid spheres are located throughout the cells and vary both in number and size relative to the age of the insect. Lipid appears to leave the gland cells of the cabbage looper after the spheres evert the apical membrane between microvilli. Gland cells of the three species contain oval to elongate microperoxisomes. In the cabbage looper, lipid spheres are surrounded by microperoxisomes except when the spheres are present in the eversions of the apical membrane.

The large lipid spheres released from gland cells of the cabbage looper are stored within the cuticle as lipid deposits. These lipid deposits appear to move to the surface of the insect by tubular structures which differ from epicuticular filaments. Hence, it is

suggested that these structures consist, at least in part, of the pheromone. No such modifications of gland cuticle were observed in either the spruce budworm or the tussock moth.

The basal lamina underlying cabbage looper gland cells is unusual and differs from that underlying gland cells of the other two species as well as that underlying unmodified non-glandular epidermal cells. Part of this unusual basal lamina is formed from the contents of granules secreted by granular haemocytes. Ultrastructural evidence indicates that material from these granules is phagocytosed by gland cells. Injection of compounds thought to be precursors suggests that production of the pheromone within gland cells of the cabbage looper occurs by a conversion of pre-existing compounds rather than de novo biosynthesis.

The defensive secretion from the red-humped caterpillar, Schizura concinna (Notodontidae) was analyzed and shown to contain n-decyl acetate. The defensive gland contains four types of cells, distinguishable ultrastructurally. The possible functions of these cells in the production of the acetate are discussed and compared with the function of gland cells in the production of the sex pheromone of the cabbage looper.

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LIST OF ABBREVIATIONS

aa - anterior apophysis

af - apical folds

AG - anterior gland

an - anus

bi - basal involutions

bm - basal lamina

bl - layer 1 of basal lamina

b2 - layer 2 of basal lamina

br - branch of lipid deposit

c - cuticle

ce - cuticular extensions

cl - cuticular lipid

cm - cuticle modified for muscle attachment

co - core of microvilli

cop - copulatory opening

cu - cuticulin

cv - coated vesicle

de - dense epicuticle

dm - dense material

dp - dense plaque

ec - epidermal cells

ee - extensions of epidermal cells

ef - epicuticular filaments

en - lamellate endocuticle

f - filamentous structure

fl - filaments in defensive gland cuticle

G - Golgi complex

ga - gaps between layers 1 and 2 of basal lamina

gc - gland cell

gd - glycogen deposit

gh - granular haemocyte

gl - gland

gr - granules in granular haemocytes

Gu - gut

Gv - Golgi complex coated vesicles

h - haemocoel

HC - head capsule

he - hemidesmosome

i - unmodified intersegmental membrane

ic - inner cuticulin

IN - interglandular neck

is - intercellular space

j - junctional region

L - gland lumen

ld - lipid deposit

li - lipid

lt - lipid tubules

m - mitochondrion

mb - microbodies

mc - muscle

me - mesocuticle

mf - microfibrils

mo - membranous organelle

MT - microthorax

mt - microtubules

mv - microvilli

mvb - multivesicular body

n - nucleus

N - nucleus of haemocyte

oc - outer cuticulin

od - oval depression

o - ovipositors

ov - ovipore

p - pore

pc - pore canal

pa - posterior apophysis

PG - posterior gland

pg - protein granules

po - origins of pore canals

PT - prothorax

rer - rough endoplasmic reticulum

ro - reorganized portion of b=2 within basal involutions

ser - smooth endoplasmic reticulum

scer - smooth cisternal endoplasmic reticulum

ster - smooth tubular endoplasmic reticulum

te - termination of re-organized portion of layer 2

to - trough

tr - trachea and tracheole

ts - tubular structures

tu - tubules

uc - unmodified cuticle

ue - uneven layer on surface of outer cuticulin

Numerical symbols

1 - muscle 1

1p – muscle 1p

2 – muscle 2

2p – muscle 2p

3 – muscle 3

3p – muscle 3p

4 – muscle 4

5 – muscle 5

7 – seventh tergite

8 – eighth tergite

INTRODUCTION

Many organisms synthesize and release substances into the environment for the purpose of communicating with organisms of the same or different species. This process is known as chemical communication and the compounds conveying the message are semiochemicals (Law and Regnier, 1971). Intraspecific semiochemicals include sex pheromones which, after release, cause in the opposite sex, an immediate behavioural response equated with sexual activity. Interspecific semiochemicals include defensive secretions which, after release, benefit the emitting organism by affording it a measure of protection from predators. The terminology associated with such airborne semiochemicals is presented in Appendix 1.

The sex pheromones of female moths and the defensive secretions of larval Lepidoptera are released from exocrine glands. In the present study sex pheromone production and release have been examined in the eastern spruce budworm, Choristoneura fumiferana (Clemens) (Tortricidae); the whitemarked tussock moth, Orgyia leucostigma (J.E. Smith) (Lymantriidae); and the cabbage looper, Trichoplusia ni (Hübner) (Noctuidae). Also included are aspects of the defensive gland and its secretion in the red-humped caterpillar, Schizura concinna (J.E. Smith) (Notodontidae). This last study is included because the defensive secretion is shown, herein, to contain a compound, the chemical structure of which closely resembles that of the sex pheromone of the cabbage looper.

The sex pheromones of the three moths are lipids, more specifically, they all represent derivatives of fatty acids. The pheromone of the spruce budworm has been isolated from females and identified as a blend consisting of 96% trans-11-tetradecenal and 4% cis-11-tetradecenal

(Weatherston et al., 1971; Sanders and Weatherston, 1976). The pheromone of the cabbage looper has also been isolated from females and identified as cis-7-dodecenyl acetate (Berger, 1966). The pheromone of the whitemarked tussock moth has not yet been isolated from the female but evidence from field testing and laboratory bioassays indicates that cis-6-heneicosen-11-one is a component of the pheromone (Grant and Frech, 1976; Grant, 1977). This compound has been isolated from the female of closely related species, the Douglas fir tussock moth (Smith et al., 1975). The chemical structure of these compounds is not unusual as they either closely resemble or, are identical to, chemicals having similar functions in other female moths (Roelofs and Cardé, 1974).

The sex pheromone gland of female moths is situated between the eighth and ninth abdominal segments and represents a modification of the intersegmental membrane between these segments. The modification is situated dorsally, laterally or ventrally within the membranous fold. This information, obtained from the literature, has been summarised in a classification scheme (Appendix 2) which was derived from the reported observations of some 50 species representing 12 families of moths (Appendix 3).

The sex pheromone glands of the spruce budworm, the cabbage looper and the tussock moth are all dorsal modifications of the intersegmental membrane (Sanders, 1969; Shorey, 1964; Percy et al., 1971). As in all other species examined (references in Appendix 3) gland cells in these three species are modified epidermal cells. Histological observations reveal that the gland cells of the spruce budworm and the cabbage looper are columnar and contain lipid droplets whereas the gland cells of the tussock moth are goblet-shaped, and do not contain lipid droplets (Percy

and Weatherston, 1971a; Jefferson et al., 1966; Percy et al., 1971).

Lipid droplets appear in gland cells of the cabbage looper during the day preceding eclosion. The droplets increase in number, but not size, up to 1½ days after eclosion (Jefferson and Rubin, 1973). It has been suggested (Jefferson et al., 1966) that lipid droplets do not increase in number or size in gland cells of older insects. It has also been suggested (Grant, 1970) that lipid droplets do increase in size, but not number, in gland cells of older insects. By comparison, the pheromone content of the gland in the cabbage looper increases considerably from eclosion to 2 days after eclosion, reaches a maximum from 2 to 4 days after eclosion and declines in older insects (Shorey et al., 1968 and summarized in Appendix 4).

The relationship between the ultrastructure of sex pheromone gland cells and the production or release of pheromones has generally been ignored. In fact there have been very few ultrastructural studies of sex pheromone gland cells. The studies are, for the most part, incomplete and often contain micrographs in which the organelles do not illustrate ultrastructural features characteristic of good fixation. Insects in which the gland cells have been studied include Bombyx mori (Bombycidae) (Steinbrecht, 1964a; Waku and Sumimoto, 1969); Plodia interpunctella (Pyralidae) (Smithwick, 1970). Diatraea saccharalis (Pyralidae) (White et al., 1973) and Agryotaenia velutinana (Feng and Roelofs, 1977). The gland cells of Bombyx mori have been most carefully examined. The results of this study indicated that, although the gland cells form the discrete lipid droplets contained within them, the lipid droplets probably do not represent the pheromone (Steinbrecht, 1964a, b).

The ultrastructure of the cuticle of the gland and its relationship to sex pheromone production and/or release has been examined only in Bombyx mori (Steinbrecht, 1964a). Ultrastructural observations of glands from insects of various ages resulted in the proposal that diffusion of pheromone from the cells and across the cuticle occurs continuously. This diffusion is maintained by evaporation of pheromone from the gland surface. The suggestion was also made that filamentous structures observed in the cuticle might be involved in the diffusion. (These filamentous structures are structurally comparable to the epicuticular filaments described by Filshie (1970a, b).)

The function of entire sex pheromone glands in the formation of the pheromone has been studied in experiments designed to determine the biosynthetic pathways of pheromone production in Bombyx mori. Sodium palmitate, labelled with C^{14}, was injected into late pupae and the sex pheromone (trans-10-cis-12-hexadecedien-1-ol) was isolated from adults and shown to carry the C^{14} label (Inoue and Hamamura, 1972).

Although the structural features of sex pheromone glands of female moths have not been completely elucidated, these glands have been examined in much greater detail than have the defensive glands of the larvae. Within the family Notodontidae, larvae of many species have a defensive gland located ventrally in the prothorax. Morphological descriptions have been reported for a few species. These species include Cerura vinula and Notodonta anceps, where the gland is a simple pouch (Hintze, 1969), and Schizura concinna (Detwiler, 1922), Heterocampa manteo and H. latitarsus (Eisner et al., 1972), where the gland consists of two distinct parts.

The defensive secretion is stored within the lumen of the gland and

is sprayed in the direction of a predator when the insect is disturbed (Herrick and Detwiler, 1919; Munro et al., 1962; Eisner et al., 1972). The secretion of many species, including Schizura concinna, contains formic acid (Poulton, 1887; Maloeuf, 1938; Eisner et al., 1961; Schildknecht and Schmidt, 1963; Hintze, 1969; Templado and Dorado, 1970; Eisner et al., 1972). In at least one species accessory aliphatic compounds (acyclic ketones) also occur (Eisner et al., 1972).

Histological studies of the defensive gland of three species have demonstrated that gland cells on the surface of the gland are separated from the lumen by a layer of cuticle. The species studied were Schizura concinna (Detwiler, 1922) and Cerura vinula and Notodonta anceps (Hintze, 1969).

The ultrastructure of defensive gland cells in notodontid larvae has not been studied. However, there has been an ultrastructural study of gland cells in dorsal, eversible defensive glands of larvae of papilionid butterflies (Crossley and Waterhouse, 1969). The defensive gland cells of the papilionid larvae do not contain the cuticular ductules which are found in defensive gland cells of other arthropods (Eisner et al., 1964; Filshie and Waterhouse, 1968; Happ and Happ, 1973).

The present study encompasses a comparison of sex pheromone gland cells in female moths of three species and a defensive gland in a notodontid larva. The comparative study progressed from the whole glands to the ultrastructural changes which are related to the production and release of secretory products.

MATERIALS AND METHODS

The spruce budworm (Choristoneura fumiferana), the whitemarked tussock moth (Orgyia leucostigma) and the cabbage looper (Trichoplusia ni) were reared in the laboratory on a synthetic diet (Grisdale, 1973). Larvae and pupae of the spruce budworm and the tussock moth were kept at 24°C and 55 - 60% humidity. Adults were kept at ambient temperature except where noted. All stages of the cabbage looper were maintained at 27°C and 50% humidity. The red-humped caterpillars (Schizura concinna) were collected from apple trees on St. Joseph Island, Ontario. They were maintained in the laboratory on apple foliage at ambient laboratory conditions.

Whole insects were photographed with a Nikon F camera and a 70 to 150 mm Macro-Zoom lens.

Whole Mounts

Whole insects were fixed by injection of either Carnoy's Fluid (Humason, 1967) or 70% ethanol. Fixation was for one hour, during which the tissues hardened, then the ventral surface of the insects were opened to expose the dorsally-situated glands and the associated muscles. The gut and other organs in the region were carefully removed and the last four abdominal segments separated from the body. Scales were removed from each segment. The terminal segments were usually bisected antero-posteriorly into left and right portions before staining.

Tissue from insects which were fixed with 70% ethanol was

immediately stained with Grenacher's borax carmine (Humason, 1967). The staining times suggested by Humason were not suitable for this tissue and the following schedule was devised:

1) Stain in borax carmine solution = 5 minutes.

2) Add 4 drops concentrated hydrochloric acid (until red precipitate occurs) and continue staining overnight at room temperature.

3) Differentiate stained tissue with 3% concentrated hydrochloric acid in 70% ethanol.

4) Dehydrate in ethanol.

5) Clear in benzene; mount in Permount.

Tissue from insects which were fixed with Carnoy's Fluid was stained with the Feulgen technique as outlined by Bronskill (1970).

Whole mounts were viewed and photographed using a Leitz Orthoplan microscope.

Red-humped caterpillars were injected with a mixture of 95% ethanol and glacial acetic acid (3:1). After one half an hour the insects were opened with a lateral incision to expose the defensive glands. A replete defensive gland occupies a large portion of the larval thorax so care was taken, when removing cuticle and adjacent organs, not to damage the reservoirs or associated muscles. The head and thorax with the gland were separated from the abdomen and stained with Grenacher's borax carmine (Humason, 1967) following the modified procedure.

The structure of the gland was deduced both from whole mounts and dissected unstained specimens.

Histochemical Tests

Glycogen. For the histochemical determination of glycogen in the

spruce budworm pheromone gland, tissue was obtained from insects which had been injected with Carnoy's Fluid (Humason, 1967). Following fixation, glands were dehydrated in ethanol, cleared in benzene and embedded in paraffin (m p. 58°C) and sectioned at 4 - 6 µm. The sections were subjected to the Periodic-Acid-Schiff reaction to demonstrate glycogen as outlined by Humason (1967). Sections treated with the enzyme diastase which removes glycogen were used as controls. A second control series was used from which the periodic acid was omitted.

Lipids. In order to detect lipids within gland cells and cuticle of the spruce budworm and the cabbage looper some sections were stained with Sudan Black B or Oil Red O.

Glands were dissected from adult spruce budworm which had been injected with 10% formalin and were embedded in water soluble wax (Carbowax 1540, Union Carbide). Sections 1 - 3 µm thick were stained with ethanolic Oil Red O for 5 minutes following which they were washed with distilled water and mounted in glycerin jelly (Humason, 1967).

Glands were dissected from adult cabbage loopers which had been injected with 5% glutaraldehyde in 0.05 M cacodylate buffer. After 2 to 4 hours in fixative, the glands were rinsed well in distilled water and mounted in D.C.T. Compound (Tissue-Tek II) for cryomicrotomy. Frozen sections, 12 to 14 µm thick were washed in distilled water to remove the mounting medium and then stained for 5 minutes with either Sudan Black B (dissolved in ethylene glycol) or ethanolic Oil Red O. Sections stained with Sudan Black B were differentiated in ethylene glycol while those stained with Oil Red O were differentiated in distilled water. Both were mounted in glycerine jelly.

Scanning Electron Microscopy

The sex pheromone glands of adult spruce budworm were extruded by applying pressure dorsoventrally to abdominal segments six and seven with a dental matrix retainer. The abdominal tip was excised and immediately affixed to a copper specimen holder by conductive silver paint. The specimens were examined with a Jeolco JSM-II scanning electron microscope, operated at an accelerating voltage of 25 kev.

Transmission Electron Microscopy

The ages of laboratory-reared insects used in the ultrastructural studies are accurate to within one hour of the time of eclosion except where noted. For very young adults of the cabbage looper, up to 18 hours after eclosion, the age at fixation is accurate to ± 5 minutes. Ages of late pupae were determined by selecting several in which pigmentation of the cuticle appeared to be similar. Some of these pupae were killed and fixed. Their age is given as the time to eclosion as observed for their counterparts.

Proper fixation of cells in sex pheromone glands and defensive glands is difficult to attain because both types of glands are shaped like a sac and lined with cuticle. The glands thus tend to float on the surface of the fixative.

The sex pheromone glands have a tendency to trap the air between them and the ovipositors. This problem can be overcome if particular care is taken in dissections to assure that only the gland and perhaps some soft tissues, i.e., no extraneous cuticular regions, are removed for fixation. A further problem is encountered in the dissection of the gland in the tussock moth. There, a dense network of tracheoles

underlies the cells; this network must be removed in its entirety before cells can be properly fixed. These problems were not encountered when removing and fixing unmodified intersegmental membranes (for use as controls).

In the case of the defensive gland, neither proper fixation nor proper infiltration of the embedding medium occurs if the gland is fixed whole. Therefore, in this study, each gland, after preliminary fixation, was dissected into smaller portions. Each portion was assigned a number indicating its position within the gland (see diagram of gland in Fig. 82).

With this technique for identification of distinct regions of the gland, the ultrastructure of representative cells from six separate regions of the gland could be determined.

Killing and initial fixation of the insects were accomplished by injection of cold 5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.3) containing 2% sucrose and 0.01 M calcium chloride (Caulfield, 1957) into the abdomen of insects. The glands were then removed and fixed in fresh ice-cold 5% glutaraldehyde for 6 to 12 hours. Tissues were post-fixed for 4 to 12 hours at 10°C with 1% osmium tetroxide in 0.05 M cacodylate buffer containing 4% sucrose. Glands were stained *en bloc* with 2% hot aqueous uranyl acetate (Locke *et al.*, 1971), dehydrated in ethanol and embedded in Araldite (Cargille Ind., New Jersey, U.S.A.) (Hayat, 1972).

Preliminary screening of the tissue for proper fixation was accomplished by examination of thick (1 µm) sections cut with a glass knife on a Reichert OMU2 or OMU3 ultramicrotome. These sections were placed on glass slides, and stained for 30 seconds with 1% toluidine blue

solution (pH 11) containing borax. Toluidine blue at this pH stains cells metachromatically and stains lipids green (DeMartino et al., 1968). The sections were destained with distilled water, dried by heating and mounted in Permount.

Thin sections were cut with a diamond knife on a Reichert OMU2 or OMU3 ultramicrotome. The sections were examined and photographed without surface staining. For studies of the spruce budworm gland, a Philips 300 electron microscope operated at 80 kv was used. For studies of glands from the cabbage looper, tussock moth and the red-humped caterpillar, a Hitachi HUI2A electron microscope was used. It was operated at 100 kv for observation and photography of sections of the cabbage looper and tussock moth gland cells and at 75 kv for the red-humped caterpillar gland cells.

Solvent extractions. The following variations of the routine fixing and embedding protocols carried out on sex pheromone glands were done in order to determine their effect on the ultrastructure of the gland cuticle:

a) Initial fixation with osmium tetroxide and omission of glutaraldehyde.

b) Prior to fixation with osmium tetroxide, excised glands or whole insects were extracted overnight at room temperature with the following lipid solvents: hexane, methylene chloride or diethyl ether. Glands were dissected from whole insects before fixation.

c) Prior to fixation with osmium tetroxide, excised tussock moth glands were extracted with hexane by dipping the glands in the lipid solvent. The extract was subsequently used to obtain positive mating responses from adult male moths (Grant, 1975).

d) Prior to initial fixation with either glutaraldehyde or osmium tetroxide, excised cabbage looper glands were extracted overnight at room temperature with hexane or

chloroform/methanol (3:1). Before these tissues were fixed in glutaraldehyde they were given a brief rinse in methanol to remove the hydrophobic lipid solvents.

DAB reaction for catalase. Insects were injected with ice-cold 2.5% or 5% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.3 containing 2% sucrose and 0.01 M calcium chloride (Caulfield, 1957). Glands were removed and fixed for 2 to 4 hours on ice. The tissue was then given two quick rinses in distilled water before being placed in the DAB medium.

For spruce budworm gland cells the technique as modified by Locke and McMahon (1971) was followed. This medium contains 10 ml 0.05 M propanediol buffer, pH 9.0, 3 mg of diaminobenzidine (DAB) free base and 0.2 ml 3% hydrogen peroxide. The reaction was carried out on a shaker at room temperature for about 12 hours. This technique, however, did not give consistent results with gland cells from the tussock moth and the cabbage looper. Therefore, the tissue was reacted in the incubation medium as outlined by Novikoff et al. (1972). This medium contains 20 mg DAB-tetrahydrochloride, 9.3 ml of 0.05 M propanediol buffer pH 9.0 (pH adjusted to 9.7) and 0.2 ml 2.5% freshly prepared hydrogen peroxide. The tissue was incubated at 37°C for 3 hours with the medium being changed every hour and the vials agitated every 15 minutes.

The following controls were used: (a) the omission of hydrogen peroxide from the medium (b) the addition of 0.5 ml of 0.1 M KCN to the medium to inhibit mitochondrial staining (c) the addition of 0.03 M 3-amino-1,2,4-triazole to the medium to inhibit microperoxisome staining.

After reaction in the DAB medium the tissue was rinsed in distilled

water, post-fixed in 1% OsO_4 for 4 to 12 hours at 10°C, embedded in Araldite. Sections were collected on uncoated grids and observed in the microscope without further staining.

Biosynthetic Studies

Adult cabbage looper moths, within an hour post-eclosion, were injected in the abdomen with 8 µl of C^{14}-labelled compounds and left at room temperature for either 8 hours or 3 days. The second interval (3 days) was chosen because it has been shown that the maximum amount of pheromone can be extracted from glands of insects at 2 and 4 days post-eclosion (Shorey et al., 1968).

The labelled compounds injected into the adults were Na acetate-C_1^{14}, Na acetate-C_2^{14} or lauric acid-C_1^{14} (Applied Science Lab. Inc., State College, Pennsylvania). Sodium acetate were dissolved in distilled water and lauric acid was dissolved in 50% ethanol. To calibrate the amount injected per insect, 4 µl of each compound were placed on small squares of filter paper which was put in 13 ml of scintillation cocktail and the activity determined in disintegrations per minute (dpm). (For amounts, see Results, Table II).

Autoradiography. After incubation, injected insects were fixed and embedded as in the standard technique, already mentioned (p. 10) except they were not stained en bloc with uranyl acetate. Sections, 1 µm in thickness, were placed in distilled water on glass slides and dried by heating.

The sections were coated with Ilford K5 emulsion as described by Rogers (1967) and allowed to dry in the dark for 2 hours. The slides were then placed in Coplin jars containing liquid scintillation cocktail

(0.1 g POPOP and 5.9 g PPO in 1.1 scintillation grade toluene). The jars were wrapped in aluminum foil and held at -20°C. The exposure periods were 2 hours, 8 hours, 1 day, 2 days, 4 days and 6 days. After exposure the slides were brought to room temperature, developed in D-19 and fixed with Kodak Hypofix.

This technique involving the use of liquid scintillation cocktail was utilized as it reduces exposure time from weeks to days. In addition, the cocktail, which acts as a proton multiplier, allows low levels of radioactivity to be detected (Przybylski, 1969; Retnakaran, 1971, 1974).

Extraction and scintillation counting. Twenty insects were injected and left at room temperature for 8 hours (10 insects) or 3 days (10 insects), after which time they were separated into groups of five. Just prior to dropping into a lipid solvent each insect was dissected into three parts: 1) head, thorax and wings (htw), 2) abdomen (abd), and 3) gland and ovipositors (gl). Thus there were two replicates for each of the two incubation times with each replicate consisting of 5 htw or 5 abd or 5 gl.

After extracting lipids overnight at room temperature, the solvent was removed and placed in vials containing 10 ml of scintillation cocktail. To the remaining tissue was added 3 ml NCS solubilizer (Amersham Searle Corp., Oakville, Ont.). This was left at 50°C for 5 days to extract any non-lipid labelled material. Afterwards, any remaining tissue was removed and 10 ml of scintillation cocktail added to the NCS-extract. All vials were placed in a Beckman LS9000 scintillation counter, counted for 10 minutes and radioactivity reported in dpm.

Background radioactivity was determined using uninjected control insects treated exactly as experimental insects.

The solvent used to extract lipids was chloroform/methanol (3:1). Chloroform/methanol is known to extract sterol esters, triglycerides, sterols, fatty acids and esters, phospholipids and the lipids from lipoproteins (Burchfield and Storrs, 1962).

Chemical Analyses of Defensive Secretion

The defensive secretion from 33 defensive glands was used in these studies. Larvae of the red-humped caterpillar were killed by freezing, the entire glands dissected out and separated into anterior and posterior glands. Each gland was then placed on a 5 mm square of filter paper and the secretion carefully squeezed out. The gland was removed from the filter paper and the secretion was extracted from paper with methylene chloride. The methylene chloride extract was analysed by gas liquid chromatography. Five µl of each solution was injected into a Perkin Elmer 990 gas chromatograph equipped with a 6' x 1/8" O.D. stainless steel column and a flame ionization detector. Two different columns were used for the analysis. The polar column contained 10% Carbowax 20M on Chromosorb W 80/100 mesh and was operated at 175°C with a helium flow rate of 40 ml/minute. The non-polar column had 5% OV-1, on Chromosorb W 80/100 mesh and was operated at 130°C with a helium flow rate of 40 ml/minute. Mass spectral analysis was carried out using a Hitachi RMS 4 mass spectrometer coupled directly to the gas chromatograph via a Finnigan glass jet separator.

n-Decyl acetate was prepared by reacting n-decanol with an acetic anhydride/pyridine reagent (acetylation kit) (Applied Science, State

College, Penn.). Aliquots of the reaction mixture were used directly for gas chromatographic and mass spectral analysis.

RESULTS

Morphology, Musculature and Eversion of Sex Pheromone Glands

Morphology of Glands

Choristoneura fumiferana: A female spruce budworm, when calling the male, exposes its sex pheromone producing gland by flexing the abdomen ventrally and evaginating the modified intersegmental membrane which then appears as a dorsal saddle between the eighth and ninth abdominal segments (Fig. 1a). Thus, in a resting insect the gland appears as a dorsal sac underneath the eighth tergite.

Trichoplusia ni: A female cabbage looper moth, when calling, raises its abdomen from the substratum (Fig. 1b). The eighth abdominal segment, normally telescoped within the seventh segment, is protruded and flexed dorsally. The glandular membrane is everted to form a white, bulbous saddle. Thus, when inverted, this gland is also a dorsal sac.

Orgyia leucostigma: A female whitemarked tussock moth is wingless; when it calls the male it does so with the last few abdominal segments slightly raised and with a rhythmic protraction and retraction of these segments. The gland is also a modification of part of the dorsal intersegmental membrane between abdominal segments 8 and 9 (Fig. 1c). However, when the segments are protruded the gland is represented as a rectangular dorsal field which is not everted as in the other two insects. In the resting insect the gland is a crescent-shaped dorsal fold (Figs. 2a, b).

The surface of the spruce budworm gland , as revealed by scanning electron microscopy, exemplifies surface modifications differentiating

these modified intersegmental membranes from unmodifie: or non-glandular intersegmental membranes (Fig. 3). A glandular membrane consists of an anterior portion possessing cuticular projections, or 'spikes' (Figs. 3a, c) and a posterior portion without spikes (Figs. 3a, d). These two portions can be distinguished on both partially everted and completely everted glands. An unmodified or non-glandular intersegmental membrane is completely devoid of 'spikes' (Figs. 3b, e). Pores, larger than 150Å in diameter, cannot be discerned on any of the surfaces. Thus the function of 'spikes', as observed on the spruce budworm gland, is in all probability, to retain the pheromone on the surface of the inverted gland and to retard the immediate evaporation of all the pheromone from the surface of the everted gland.

Musculature of Glands

Within insects, muscles are often attached to internal, tubular invaginations of cuticle (= apophyses) (Snodgrass, 1935). Terminal abdominal segments of a female moth contain one pair each of anterior apophyses and posterior apophyses (Matsuda, 1976).

In the spruce budworm, the whitemarked tussock moth and the cabbage looper, the anterior apophyses originate from the anterior borders of the eighth tergite either dorsolaterally (Fig. 4a, b, d and Fig. 6) or laterally (Fig. 4c and Fig. 6). The apophyses proceed anteriorly and fork immediately anterior to the eighth tergite in the spruce budworm and the tussock moth. The ventral arm of each apophysis surrounds the body to form part of the copulatory opening while the dorsal arm continues anteriorly to terminates freely in the seventh segment (double arrows in Fig. 4b and Fig. 6a, c). In the cabbage looper the

anterior apophyses are straight and do not fork (double arrows in Fig. 4c and Fig. 6b).

The posterior apophyses in all three species originate from the ovipositors (encircled area in Fig. 5a, b, c). Immediately anterior to this they form part of the lateral cuticular wall of the sex pheromone glands (arrows in Fig. 5a, b, c). The posterior apophyses lie mediad to the anterior apophyses within the insects and terminate anteriorly in the 8th segment of the tussock moth (Fig. 4d) and the 7th segment of the cabbage looper (Fig. 4c) or the resting spruce budworm (Fig. 4a). In the calling spruce budworm (i.e., with gland everted) the posterior apophyses terminate in the 8th segment and are curved rather than straight as in the resting insect.

Muscles associated with the apophyses and therefore with the pheromone glands are positioned dorsally or laterally within abdominal segments 7 and 8 in all three species. The muscles are illustrated in Figs. 2, 4, 5 and interpreted semidiagramatically in Fig. 6. There are 8 groups of these bilaterally paired muscles which, in the following description are designated as groups 1, 2, 3, 4, 5 (each member of a pair attached to an anterior apophysis) and groups 1p, 2p, 3p (each member of a pair attached to a posterior apophysis). Groups 1 and 3 consist of more than one pair of muscles in which case each pair has been assigned a letter, i.e., 1a, 1b, 1c, 3a, 3b. The musculature of the three species is similar but not identical, therefore, individual variations are listed after the general description for each group.

Group 1: These muscles originate on the anterior margin of the seventh tergite immediately posterior to the intersegmental membrane

between the 6th and 7th segments. They insert at the anterior free termination of the anterior apophyses.

Spruce budworm = A single pair of muscles. (1)[1].

Cabbage looper = Two distinct pairs of muscles. (1a, 1b)

Tussock moth = Four distinct pairs of muscles with 1a and 1b being oblique and mediodorsal and 1c and 1d being laterodorsal. (1a, 1b, 1c, 1d)

Group 1p: These muscles originate immediately posterior to the intersegmental membrane between the 6th and 7th segments and insert at the anterior termination of the posterior apophyses.

Spruce budworm - One pair of muscles which originate mediodorsally to muscle 1. (1p)

Cabbage looper = One pair of muscles which originate dorsally to muscle 1a. (1p)

Tussock moth - One pair of muscles which originate ventrolaterally to muscles 1a, 1b, 1c and mediodorsally to muscles 1d. Some of the fibers are attached to termination of anterior apophysis adjacent to muscles 1a, 1b, 1c, 1d. (1p)

Group 2: These relatively short muscles originate at the posterior laterodorsal margins of the seventh tergite and insert on the anterior apophyses.

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1. The numbers in brackets correspond to the labelling of each muscle as observed in Figs. 2, 4, 5 and 6.

Spruce budworm - One pair of muscles which insert medially at the fork of the apophyses. (2)

Cabbage looper - One pair of muscles which insert externally at the terminations of the apophyses. (2)

Tussock moth - These muscles are not present. Muscles originate from a similar position but their insertion is on the laterodorsal cuticle in the posterior half of the seventh segment.

Group 2 p: These originate at the posterior laterodorsal margins of the eighth tergite and insert externally at the anterior terminations of the posterior apophyses.

Spruce budworm - One pair of muscles with no variation. (2p)

Cabbage looper - One pair of muscles with no variation. (2p)

Tussock moth - One pair of muscles with no variation. (2p)

Group 3: These muscles originate on the anterior apophyses and insert lateroventrally at the anterior edge of the ventral intersegmental membrane between the eighth and ninth abdominal segments.

Spruce budworm - One pair of muscles originating ventrally at the fork of the apophyses and insert posterior and dorsolateral to the copulatory opening. (3)

Cabbage looper - Two muscle pairs, 3a and 3b; they originate ventrally at the anterior end of the apophyses and insert lateral and posterior to the ovipore. (3a, 3b)

Tussock moth - There are no comparable muscles in this insect.

Group 3 p: These originate near the anterior ends of the posterior apophyses and run posteriorly with different insertions in each insect.

Spruce budworm - One pair of muscles which insert on the rectum.

Cabbage looper - One pair of muscles which run dorsally to insert lateral to the anus. (3p)

Tussock moth - One pair of muscles which insert ventrolaterally on the intersegmental membrane between the 8th and 9th segments immediately posterior to the ventral arms of the anterior apophyses (i.e.) insertion similar to those of group 3 in the spruce budworm and the cabbage looper but the origin differs.

Group 4: These muscles originate on the anterior apophyses and insert on the portions of the posterior apophyses which form part of the walls of the glands.

Spruce budworm - One pair of muscles which originate medially near the forks of the anterior apophyses and insert ventrolaterally on the posterior apophyses. (4)

Cabbage looper - One pair of muscles which originate externally at the anterior termination of the apophyses and insert ventrolaterally on the posterior apophyses. (4)

Tussock moth - One pair of muscles which originate externally and anteriorly to the forks of the apophyses and insert externally on the posterior apophyses. (4)

Group 5: These originate at the anterior terminations of the anterior apophyses and insert ventrolaterally at the posterior margin of the seventh segment.

Spruce budworm - One pair of muscles which insert anterior and dorsal to the copulatory opening. (5)

Cabbage looper - The muscles are not present in this insect.

Tussock moth - One pair of muscles which insert anterior and dorsal

to the copulatory opening. (5)

Other muscles: These may not be as closely related to eversion or protrusion of the gland but nevertheless may play a role.

Spruce budworm = Muscles which originate on the anterior apophyses and terminate on the gut and common oviduct.

Cabbage looper - Muscles which originate on the anterior apophyses and terminate on the common oviduct.

- Dorsal muscles which originate near the intersegmental membrane between the 6th and 7th segments and insert near the membrane between the 7th and 8th segments.

Tussock moth - Dorsal muscles which originate near the intersegmental membrane between the 6th and 7th segments and insert near the membrane between the 7th and 8th segments.

Terminology of muscles

The muscles of the sex pheromone glands in all three species are associated with the two pairs of apophyses which have as their origin tergites of the successive segments, 8 and 9. Therefore, these muscles represent some of the body musculature from two abdominal segments and are analogous to those groups of muscles as listed and described by Snodgrass (1935). The groups to which the muscles of the pheromone glands belong include the internal dorsals, the reversed external dorsals and the laterals (summarized in Table I).

The internal dorsals are composed primarily of longitudinal fibers of segmental length and are attached on successive intersegmental folds or may shift their origins to the postcostal regions of the folds. Functionally, they act as abdominal retractors (Snodgrass, 1935). In

the present study groups 1, 1p and 4 are internal dorsals. As abdominal retractors they should also cause inversion of the glands.

Reversed external dorsals are short longitudinal muscles seldom of segmental length which often become oblique. When their origins are transposed to the posterior margins of the terga they become functionally antagonistic to the internal dorsals and become abdominal protractors (Snodgrass, 1935). In the present study, groups 2 and 2p are reversed external dorsals whose origins are located on the posterior margins of terga. Therefore, these muscles as abdominal protractors may cause eversion (or protrusion) of the pheromone glands.

The laterals are usually intrasegmental in position and tergosternal in attachment but they may cross obliquely from one segment to the next. Functionally, they act as compressors of the abdomen (Snodgrass, 1935). In the present study groups 3, 3p and 5 are laterals. By acting as lateral compressors of the abdomen, they may cause eversion, or protrusion of the glands.

Ultrastructure of Developing and Mature Gland Cells

Sex pheromone gland cells in all three species are modified epidermal cells of an intersegmental membrane (Jefferson et al., 1966; Percy et al., 1971; Percy and Weatherston, 1971a). For the purpose of the ultrastructural study described herein, gland cells are divided into groups according to their stages of development to facilitate comparison to each other and to the ages of the insects. Developing cells represent both gland cells and unmodified epidermal cells and overlying cuticle as observed during the time the characteristics of the adult insects are beginning to appear. Maturing cells represent gland cells and overlying

cuticle during the time when the characteristics of the adult have appeared but the insects are not yet releasing much sex pheromone, i.e., shortly before eclosion or during the first few hours after eclosion. Mature (or adult) cells represent gland cells and their overlying cuticle as they appear at maturation and during the time when insects are most attractive to males. The structure of unmodified epidermal cells and overlying cuticle in mature adults is presented as a comparison.

Developing cells

In pupae 1 - 2 days before eclosion, characteristics of the adult insect are appearing and both developing gland and unmodified epidermal cells are columnar and similar in structure. Microvilli are present as extensions of the apical membrane (Figs. 7, 9, 11). Microvilli are short, with dense plaques at their tips and in the spruce budworm (Fig. 7) and the tussock moth (Fig. 9) show very few circular cross sections thus indicating that, at this stage apical folds occur as well as true microvilli. Microvilli are more numerous in the cabbage looper (Fig. 11). Coated vesicles and multivesicular bodies are seen near the bases of the microvilli. The cytoplasm contains free ribosomes and rough endoplasmic reticulum. In the apical region above the nucleus there are mainly short disorganized profiles (Figs. 7, 9) while longer profiles prevail in the basal region (Figs. 8, 10). Golgi complexes are present in both the apical and basal regions, as are microtubules. Mitochondria are very numerous. Microbodies are infrequent. Lipid deposits, irregular in shape, are present only in the gland cells of the cabbage looper (Fig. 11). At 36 hours before eclosion these are few in number and may

be located in any region of the cell (Fig. 11).

A thin amorphous basal lamina underlies the developing gland cells and epidermal cells of the spruce budworm and the tussock moth (as illustrated for the adult cells in Figs. 19, 21). However, even at 36 hours before eclosion the basal lamina underlying the gland cells of the cabbage looper has distinctive characteristics (Fig. 11). This basal lamina is thick (up to 5 μm) and usually exhibits two distinct layers. Layer 1 is amorphous and lies next to the basal plasma membrane. Layer 2 is distinctly banded and lies next to the haemocoel.

The soft untanned cuticle of the developing cells contains several lamellae of endocuticle (Figs. 9, 11, 12). The parabolic appearance of layers as observed in the electron microscope is produced by helicoidally arranged microfibrils and is characteristic of lamellate endocuticle (Neville and Luke, 1969).

The endocuticle underlies a thin layer of dense epicuticle which in turn underlies even thinner inner and outer cuticulin (Fig. 12). The total thickness of the cuticle is extremely variable because of mammiform eversions on both unmodified and gland cuticles and the presence of epicuticular projections, 'spikes' on spruce budworm (Fig. 3c, 19) and cabbage looper gland cuticles (Fig. 25).

Traversing the cuticle of all three species are epicuticular filaments which are located in clear spaces free of chitin-protein microfibrils. These clear spaces represent pore canals which in soft cuticle are not as well-defined as in hard cuticle (Neville *et al.*, 1969). The pore canals, at low magnification, are only visible because they contain the epicuticular filaments. However, even these ill-defined pore canals follow the helicoidal arrangement of the chitin-protein microfibrils.

The pore canals originate very close to the microvilli, their contents possibly consisting in part of the fibrillar fuzz on the microvillar surface (Fig. 13). A pore-canal filament as observed in other types of cuticle is not seen, although a somewhat similar structure may be present. This structure, filamentous and sometimes twisted, originates very close to the microvilli. It extends, however, only to the first lamella of the endocuticle. It is here that there is some indication that it is not similar to other pore canal filaments. It is also in this region that the typical structure of the epicuticular filaments is first encountered (Fig. 13).

The density of epicuticular filaments in pore canals of the endocuticle is extremely variable even within adjacent regions of the same cuticle. The filaments are tubular and variable in length (Fig. 34). They seldom penetrate the dense epicuticle singly. Beneath it they are surrounded by a clear area (continuation of pore canal?). Within the dense epicuticle are electron-lucent oval to circular areas (Fig. 12 and also Figs. 34, 41, 54). Groups of epicuticular filaments terminate at the bases of these lucent areas which form the pores seen at the surface of the inner cuticulin. It could not be determined from the present techniques whether the pores open at the surface of the outer cuticulin.

The interpretation of a generalized developing gland or unmodified epidermal cell and overlying cuticle is presented diagramatically in Fig. 14 and is representative of all three species. Its main ultrastructural features include: extensive development of rough endoplasmic reticulum located near the base and oriented

along the longitudinal axis of the cell; many coated vesicles and multivesicular bodies near the apical plasma membrane; and short, disorganized microvilli and apical folds on the apical plasma membrane. Within the pore canals of the cuticle are epicuticular filaments which consistently terminate at an oval depression opening at the surface of the inner cuticulin. The other distinguishing features exhibited only by cabbage looper developing gland cells, and not illustrated in this generalized interpretation are the lipid deposits and the unusual bilayered basal lamina.

Adult unmodified epidermal cells

Epidermal cells from unmodified intersegmental membranes and the cuticle overlying them are structurally similar in all three insects (Figs. 15, 16, 17). The height of the cells is sometimes less but never more than the thickness of the cuticle, i.e., about 5 μm. The large lobed nucleus as illustrated in Fig. 15 is flattened and 6 - 10 μm in width. Basally, the plasma membrane is deeply infolded with hemidesmosomes present at the points of apposition with the thin amorphous basal lamina (Fig. 17).

The apical surface of the plasma membrane has small projections about 0.1 μm in height. They are very small and circular cross sections are seldom observed, therefore, they are probably folds. Coated vesicles are often observed between projections (Fig. 17). The cytoplasm of these cells contains short segments of rough endoplasmic reticulum with very little smooth endoplasmic reticulum (Figs. 16, 17). Mitochondria and microtubules are also observed. At least one Golgi complex (Fig. 17) is evident in sections of cells from each species.

The cuticle exhibits the same ultrastructure as that described for developing cells with the exception that there are noticeably fewer epicuticular filaments.

The interpretation of a generalized adult epidermal cell from an unmodified intersegmental membrane is presented in Fig. 18. There are no distinct ultrastructural features of this cell which would indicate that it is engaged in secretory activity peculiar to an intersegmental membrane since the ultrastructure closely resembles that reported for epidermal cells in larvae of other insects (Locke, 1969a).

Maturation of gland cells

Gland cells begin to acquire their adult characteristics (i.e. they mature) during the day preceding eclosion in all three species. Maturation is complete by 1 hour before eclosion in the spruce budworm, by 12 to 24 hours after eclosion in the tussock moth and by 6 to 12 hours after eclosion in the cabbage looper. The following description represents observations of gland cells during maturation and includes changes which are detectable with the light microscope or at low magnifications in the electron microscope.

Spruce budworm. During the last few hours before eclosion, specific changes in maturing gland cells include the accumulation of large deposits of glycogen near the base of each cell (Fig. 19). Such deposits were identified as glycogen by a positive reaction to the Periodic-Acid Schiff test. The second specific change, detectable at low magnifications, is the appearance of many lipid spheres situated between the nucleus and basal plasma membrane. These structures are

assumed to be lipid spheres because of their positive reaction with Sudan Black B or Oil Red O and by their appearance in thin sections. After the presence of lipids was detected, a series of insects ranging in age from newly eclosed to 3 days old (EC + 3 days), were subjected to a time-series study. These insects were kept under a strict light/dark cycle, temperature and humidity regime. Sample tissues were fixed at 1 hour intervals over the 3 day period. During this period there was no variation in the size, number or distribution of lipid. This indicates that the lipid is not the actual sex pheromone or that so little of it is being used that fluctuations were not detectable in thin sections.

There are no detectable changes in the cuticle of the spruce budworm.

Tussock moth. During the 24 hours preceding eclosion the columnar shape of developing gland cells (Fig. 9, insert) changes to a goblet shape (Figs. 20, 21) characteristic of the adult cells in this species. Inward extensions of endocuticle occupy the 'neck' of each mature goblet-shaped cell (Fig. 21).

From eclosion and until EC + 12 hours the composition of the cytoplasm resembles that of a pre-eclosion insect even though the shape of the cells has changed (Fig. 20). In contrast to observations in cells from the other two species, there is no evidence of lipid or glycogen accumulation in cells from the tussock moth (Fig. 21).

The endocuticular extensions, (ce in Figs. 20, 21) around which the gland cells are clustered, represent inward projections of pore canals. Protein granules are present in the cuticular layer between the

microvillous surface of the gland and the region where lamellae of the endocuticle begin (Fig. 21). In the endocuticle small clear areas which contained the protein granules become progressively smaller and more properly resemble the pore canals over developing gland cells (Figs. 21, 41).

Cabbage looper. By the time this insect has eclosed the lipid deposits seen in developing gland cells have disappeared (Figs. 11, 22, 23 and diagrammatically, Fig. 24). In their place are lipid spheres which stain green with toluidine blue in thick sections and blue black with Sudan Black in frozen sections. In insects younger than EC + 3 hours (Fig. 24) these spheres are uniform both in size and distribution while in older insects, there is a considerable disparity in both their size and distribution. The number of lipid spheres is extremely variable from cell to cell. (Estimates of the number and size of lipid spheres per section as related to the age of the insect are given in the legend to Fig. 24.) In general, in cells from eclosion until EC + 7 hours (Fig. 22) the lipid averages less than 1 µm in diameter while in the older insects (Fig. 23) only lipid spheres in the basal part of the cell average less than 1 µm in diameter.

The cuticle overlying glands from adult cabbage loopers younger than about EC + 6 hours (Fig. 25) is very similar in appearance to that overlying developing gland cells (Fig. 11). The cuticle overlying glands from insects older than EC + 6 hours frequently exhibits structural features different from those of cuticle overlying developing and maturing gland cells (compare Fig. 25 with Fig. 26). As seen in thick sections prepared for the light microscope and stained with

toluidine blue, the endocuticle from glands of females younger than EC + 6 hours stains uniformly mauve. In sections from glands of females older than EC + 6 hours the endocuticle contains areas which are stained green. These areas sometimes appear as a green band but more often appear as green (or clear) spheres in the mauve matrix of endocuticle. With this staining technique a green colour represents lipid (De Martino et al., 1968). The clear spheres in the endocuticle are interpreted as areas from which the lipid has been extracted during preparation of the tissue. As seen in the electron microscope the cuticular lipid (cl in Fig. 26) is represented by numerous electron lucent areas of variable shape, but sometimes spherical, with a diameter of 0.5 µm to 2.5 µm. They contain a fine electron dense precipitate of osmium.

Cells from glands of these older insects sometimes contain large quantities of lipid while the cuticle overlying them is relatively lipid-free. However, lipid usually is present both in the mature cell and the cuticle overlying it, with the cuticular lipid appearing just about the time the cell becomes mature.

Comparative ultrastructure of adult gland cells and cuticle

The morphological features characteristic of maturing and adult gland cells are distinctive for each species. Detailed comparisons of the ultrastructure of these cells reveal further distinctive characteristics, as well as some similarities, among organelles as they appear within gland cells of each species.

Spruce budworm

Cell structure. Whether the female remains a virgin or mates there

is little visible alteration in the ultrastructure of the pheromone gland cells from eclosion up to EC + 7 days.

As outlined by the plasma membrane, the apical region possesses extensive well-organized microvilli. In the upper one-quarter of apposing lateral membranes, junctional areas prevail. Beneath this region the lateral membranes are a variable distance apart, and junctions are rarely observed. The entire length exhibits extensive interdigitation. The basal plasma membrane is also highly folded but the basal lamina does not follow the folds (Figs. 19, 28). The basal lamina is not present in the lateral spaces between cells nor does it surround the tracheoles which penetrate this space (Fig. 28).

The cytoplasm between the nucleus and base of the cell is characterized by extensive rough endoplasmic reticulum (Figs. 27, 28). The rough endoplasmic reticulum does not appear as well-organized as in the developing cell. Some smooth tubular endoplasmic reticulum is present (Fig. 27). Glycogen can occupy a large part of the basal area (Fig. 19). Lipid spheres are often found in close association with mitochondria (Figs. 27, 28). Coated vesicles are occasionally seen on basal and lateral membranes (Fig. 28).

Between the nucleus and the apical membrane the cytoplasm is characterized by the presence of smooth tubular endoplasmic reticulum (Fig. 29). Rough endoplasmic reticulum and free ribosomes, when present, are situated near the lateral cell membranes (Fig. 30). In addition, at these lateral cell membranes there are apposing cisternae of rough, or smooth endoplasmic reticulum. The rough endoplasmic reticulum, in these cases, lacks ribosomes on the sides facing the plasma membrane. A further characteristic of this region is the number of

microbodies, about 0.1 μm to 0.2 μm in diameter, which are closely associated with the endoplasmic reticulum (Fig. 31). They are oval to circular in section and their contents are slightly granular. Occasionally, within the contents, there is a small area which exhibits an electron density slightly greater than the remainder of the contents (open arrow, Fig. 30).

Immediately beneath the microvilli are multivesicular bodies and coated vesicles (Fig. 29). Mitochondria are numerous in this region but they do not penetrate the microvilli. Each microvillus contains a tubule of smooth endoplasmic reticulum or 'core' (Figs. 29, 32, 33). The tubule of smooth endoplasmic reticulum penetrates each microvillus for almost its entire length as evidenced by the fact that microvilli without 'cores' are seldom seen (Figs. 32, 33). Microfibrils stretch horizontally from the 'core' to the lateral membranes of a microvillus. These microfibrils probably function as a support for a microvillus. The structure of microvilli is interpreted diagrammatically in Fig. 35c.

Cuticle structure. The structure of cuticle overlying gland cells is identical to that described for developing and unmodified epidermal cells and does not change during the time period studied (Figs. 12, 13, 34 and interpreted in Fig. 35b and c). There are 8 to 10 lamellae of endocuticle each with a thickness of 0.5 μm (Figs. 12, 13). The twisted, filamentous structure observed near the tips of the microvilli has a diameter of 400 to 500Å (Fig. 13). In the remainder of the endocuticle, epicuticular filaments in pore canals have an outer diameter of 140Å and an inner diameter of 80Å. The filaments accumulate beneath the epicuticle (thickness 0.5 μm) and penetrate it in bunches (Fig. 34).

They terminate on oval depressions (300 to 400Å wide and 400 to 500Å deep) which form pores on the surface of the inner cuticulin (open arrows in Fig. 34, and insert in Fig. 34). The inner cuticulin (thickness 100Å) is separated from the outer cuticulin (thickness 80Å) by a clear area which is about 50Å thick. On the outer surface of the outer cuticulin there is an uneven layer which is not always well preserved in embedded material. When present it gives a fuzzy outline to the surface of the outer cuticulin (Fig. 12), i.e., the outer surface of the insect.

The interpretation of the spatial relationship of organelles within an adult gland cell and overlying cuticle is presented diagrammatically in Fig. 35. An adult gland cell of a modified intersegmental membrane is hypertrophied and columnar, in contrast to the flattened epidermal cell of an unmodified intersegmental membrane (Fig. 18). Organelles characteristic of this adult gland cell differ from those of the developing gland cell (Fig. 14) and consist of: lipid spheres located near the base; glycogen deposits located near the base; smooth tubular endoplasmic reticulum and microbodies located apically between the nucleus and well-organized microvilli. Each microvillus contains a tubule of smooth endoplasmic reticulum. The structures observed within the cuticle do not differ from those of the developing cuticle or of the cuticle of an unmodified intersegmental membrane.

Tussock moth

Cell structure. From shortly before eclosion and until the moth is about EC + 12 hours, the cytoplasm of the cells is similar in composition to that of the developing cells (Fig. 20). Some ultrastructural

changes do become apparent, e.g., smooth endoplasmic reticulum appears and the microvilli elongate and contain a tubule of this endoplasmic reticulum. From EC + 12 hours to EC + 24 hours there are very marked changes in the structure of the cytoplasm (Figs. 36 to 40, 42). These are obvious in gland cells from EC + 1 day to EC + 3 day virgin insects and mated insects (EC + 1 day). Variation occurs in the structure of individual cells of a single gland, but no variation could be related to age or physiological state of the insect.

Usually the cytoplasm of the adult or mature gland cells has extensive development of smooth endoplasmic reticulum, which is mainly cisternal. Fenestrae of the cisternae, when observed in surface view, have a diameter about 500Å (Figs. 36, 38). In appropriate sections, particularly near the bases of the microvilli, cisternae adjoin tubules of smooth endoplasmic reticulum and under these circumstances the tubules penetrate the microvilli (Fig. 42). Smooth endoplasmic reticulum is observed as a continuation of the cisternae of rough endoplasmic reticulum. In many cells rough endoplasmic reticulum is prevalent and usually well-organized. Mitochondria, microbodies and Golgi complexes are located among the smooth endoplasmic reticulum (Fig. 37). Microbodies appear as finely granulated inclusions in distensions of tubules of smooth endoplasmic reticulum and are surrounded by other tubules (Figs. 36, 37, 40).

The presence and size of microvilli are variable. The apical plasma membrane may be completely free of microvilli but when present they may be 2 to 3 μm in length (Figs. 36, 42). Microvilli are often absent in regions of cells with extensive and well-organized

rough endoplasmic reticulum or smooth endoplasmic reticulum (Figs. 37, 38).

Many cells contain an unusual membranous structure near the nucleus (Fig. 39). It is composed of membranous arrays of smooth-surfaced endoplasmic reticulum, not fenestrated and which has a diameter of 150 to 200Å. Cisternae of rough endoplasmic reticulum are continuous with those of this structure, thus, the stacks of smooth surface membranes probably represent degranulated rough endoplasmic reticulum (Fig. 40). A Golgi complex and many microbodies are located at each end of such structures (Figs. 39, 40).

Golgi complexes are very numerous and may be seen in any region of gland cells (Figs. 37, 39). The cisternae are surrounded by coated vesicles (diameter 650Å to 700Å) and secretory vesicles. Both the cisternae and the secretory vesicles may contain electron-dense deposits. Occasionally, secretory vesicles contain concentrically laminated structures resembling myelin figures. Near the Golgi complexes are also microbodies which have either elongate or circular profiles (Fig. 40).

Involutions of the basal plasma membrane penetrate the cytoplasm deeply (Fig. 39). However, a thin basal lamina (400Å) does not follow the contours of the plasma membrane.

Cuticle structure. Cuticle overlying adult gland cells is 2.5 µm to 3 µm thick and consists of several lamellae of endocuticle and a thin (0.5 µm) epicuticle (Figs. 21, 41). At the point where the inwardly projecting endocuticular extensions (expanded pore canals) are confluent with the endocuticular lamellae the usual structure of pore canals appears (Fig. 41). They contain very few epicuticular filaments

which are 200Å to 250Å in diameter and of indeterminate length. They penetrate the dense epicuticle in groups to the level immediately beneath the inner cuticulin. At this point the clear canal surrounding the filaments forms a large oval to circular depression (maximally 1000Å deep and 1000Å wide). The depressions open near the outer edge of the inner cuticulin. The inner cuticulin is about 150Å thick and is separated by about 80Å from the outer cuticulin (50Å in thickness). An uneven layer (i.e. in Fig. 41) exterior to the outer cuticulin is often absent from sections.

The interpretation of the spatial relationship of organelles within the adult gland cell and the structure of the overlying cuticle in the tussock moth is presented in Fig. 43. Adult or mature gland cells of the modified intersegmental membrane in the tussock moth are hypertrophied and goblet-shaped in contrast to the flattened epidermal cell of an unmodified intersegmental membrane (Fig. 18). Organelles characteristic of an adult gland cell of the tussock moth differ slightly from those organelles characteristic of an adult gland cell of the spruce budworm (Fig. 35). An adult gland cell of the tussock moth contains extensive development of smooth cisternal endoplasmic reticulum and lacks discrete lipid spheres whereas the gland cell of the spruce budworm contains smooth tubular endoplasmic reticulum and contains lipid spheres. In addition there is a membranous structure near the nucleus in the gland cell of the tussock moth and this is not observed in the gland cell of the spruce budworm. Furthermore, the microvilli of the tussock moth gland cell, while structurally similar to those of the spruce budworm gland cell, appear to be transitory features of a cell

in that they are not always present. The cuticle overlying the gland cell of the tussock moth differs significantly from that of the developing gland cell (Fig. 14) and that of the spruce budworm (Fig. 35).

Cabbage looper

Cell structure. The bilayered appearance of the basal lamina as observed underneath developing cells (p. and Fig. 11) is consistently present underneath gland cells in the adult (Figs. 22, 23) and absent underneath other epidermal cells of the insect (Fig. 17). The banding pattern characteristic of layer 2 of the basal lamina (Fig. 44) reappears between layer 1 and the basal plasma membrane of the gland cell as a re-organized portion (ro in Fig. 45). This reorganized portion penetrates between lateral plasma membranes and within basal infolds for a considerable distance. At its most apical portion the bands disappear in an amorphous material which is considerably more electron dense than either layer 1 or layer 2 (Figs. 44, 45, 46). Hemidesmosomes and coated vesicles occur at intervals along the entire length of this reorganized portion. Both hemidesmosomes and coated vesicles are also located along the basal plasma membrane with coated vesicles being particularly numerous during the first 12 hours after eclosion (Figs. 46, 47). (The only place where the bilayered appearance of the basal lamina is not evident is in the vicinity of tracheole penetration between gland cells. There only the amorphous inner layer (layer 1) is present (Fig. 48).)

The apical plasma membrane of the adult gland cells exhibit microvilli which contain tubules of smooth endoplasmic reticulum (Figs. 25, 49). These tubules, as illustrated for gland cells of the spruce

budworm (Fig. 29) and the tussock moth (Fig. 42) are continuous with tubules of smooth endoplasmic reticulum within the cytoplasm.

The entire cytoplasm of the adult gland cells contains considerable rough endoplasmic reticulum (Figs. 49, 50, 51). Mitochondria and Golgi complexes are very numerous, and are frequently but not exclusively observed in the vicinity of lipid spheres (Figs. 50, 52). Some lipid spheres appear free in the cytoplasm (Fig. 50) but usually they are surrounded by smooth cisternal and tubular endoplasmic reticulum and microbodies (Fig. 52). The microbodies are either spherical or elongate (Fig. 52).

The appearance, development and transport of the lipid will be discussed after the observations of the cuticular structure are presented.

Cuticle structure. The cuticle overlying gland cells is 3 to 5 μm thick with the endocuticle consisting of several disorganized lamellae (Figs. 24, 25, 26). In unmodified cuticle each lamella is about 0.5 μm thick (Fig. 17). It is difficult to ascertain the thickness in gland cuticle containing considerable lipid because the lipid breaks the normal pattern of the lamellae (Figs. 26, 53). Epicuticular filaments (diameter about 150 to 200Å) are observed around the lipid and underneath the epicuticle (thickness 0.15 to 0.2 μm) (Fig. 53). The epicuticular filaments penetrate to the inner cuticulin (thickness about 100Å) and terminate at an electron lucent oval depression (200Å wide and 300Å deep) which opens on the surface of the inner cuticulin (Fig. 54). The inner cuticulin is about 100Å thick. In very young insects (EC + 1 hour) it is separated by about 50Å from the outer cuticulin which is represented by a thin dense line. External to the outer

cuticulin is a thin uneven layer (Fig. 54). In a slightly older insect (EC + 3 hours) this outer uneven layer overlies and intermingles with tubules having a diameter about 200Å (Fig. 55). However, in most insects these tubules are not observed and are replaced by a dense unorganized region often containing osmium precipitate (Fig. 56).

Lipid formation and transport. The development of lipid spheres within gland cells of the cabbage looper is directly comparable with the development of the cells and the age of the insect (Fig. 24). Changes observed in the ultrastructural appearance of these lipid spheres, as related to the age of the insect, is reported in this section and is interpreted diagrammatically in Fig. 69 (Steps 2 to 6).

(i) Both smooth endoplasmic reticulum and Golgi complexes contribute towards the formation of lipid spheres (Figs. 51, 52, 57 to 61 and Fig. 69 (Step 2)).

Gland cells from newly eclosed females contain very few lipid spheres. They do contain tubular and many small patches of cisternal smooth endoplasmic reticulum (Fig. 51). The latter is often found near the maturing face of Golgi complexes (Figs. 52, 57). The lipid spheres in these cells and up to EC + 6 hours are small (1 µm more or less in diameter). When present they are free in the cytoplasm but covered by a 'cap' of smooth endoplasmic reticulum (Figs. 58, 60, 61). The tubular arms of the endoplasmic reticulum are distended and contain a granular material, forming organelles called 'microbodies' (Figs. 58, 60). These microbodies are interconnected by smooth tubular endoplasmic reticulum. They spiral around the lipid spheres (Figs. 52, 61, 62). Coated vesicles are very prevalent on basal plasma membranes and basal

infolds in gland cells from these young insects (Fig. 47). (Coated vesicles are known to be the sites of uptake of extracellular material (Whaley et al., 1971).) The observations suggest that materials, probably sequestered from outside the cell, is transformed into gland cell lipid primarily through the intervention of smooth endoplasmic reticulum and Golgi complexes.

(ii) Accumulation of lipid in the spheres continues as they progress from the base to the apex of the cell (Figs. 24, 49, 50, 62 and Fig. 69 (Step 3)).

When insects are older than EC + 6 hours and until at least EC + 141 hours there is a distinct separation in size classes of lipid spheres within cells (Fig. 24). Near the base they are usually less than 1 µm in diameter while near the apex they may reach 10 µm in diameter (Figs. 23, 49). Furthermore, smooth endoplasmic reticulum, microbodies and Golgi complexes remain closely associated with them, as represented for a gland cell from a young insect (Fig. 52). As spheres increase in diameter, they are seldom observed without a surrounding 'halo' consisting of circular profiles of microbodies (Fig. 62). They are also frequently surrounded by elongate and oval profiles of microbodies of varying length (Fig. 52). From such observations it has been deduced that, during the time when lipid is accumulating in gland cells of the cabbage looper, most of the spheres are surrounded by spiralling elongate microbodies. This is interpreted diagrammatically in Step 3 of Fig. 69.

(iii) The accumulation of lipid stops as the spheres reach the bases of the microvilli and move into the cuticle (Figs. 63, 70, and Fig. 69 (Step 4)).

When the spheres reach the crypts between the microvilli they lose contact with microbodies and Golgi complexes (Fig. 63). They move from the cell to the cuticle by forcing out the plasma membrane between the microvilli. Only occasional profiles of smooth tubular endoplasmic reticulum and a few ribosomes are then located between the lipid and the plasma membrane (Fig. 64). This movement would not involve the formation of new membranes, merely the use of that normally forming the microvilli.

(iv) The storage of lipid is within the cuticle but still within the confines of the cell (Figs. 65, 66, 67 and Fig. 69 (Step 5)).

Once the lipid has left the level of the microvilli and enters the cuticle it no longer has any organelles between itself and the surrounding plasma membrane (Broken arrows in Fig. 65). It remains attached to the apical plasma membrane of the cell but the attachment must be very fine or temporary because connections can only infrequently be deduced (Solid arrows in Figs. 64, 65, 66). The main storage area is roughly halfway and between lamellae of the endocuticle (Fig. 26) and is most frequently observed in cuticle from older insects (EC + 18 to 141 hours). The larger deposits branch quite frequently and the smaller branches follow the helicoidal patterns of microfibrils to the next lamella (Fig. 66). Once within the cuticle the lipid becomes intensely osmiophilic and is recognized by a dense granular osmium precipitate often not preserved in sections.

(v) A form of the lipid passes through pores in the cuticle to lie on the outer surface of the insect (Figs. 55, 67, 68, and Fig. 69 (Step 6)).

Immediately beneath the dense epicuticle lipid surrounded by plasma

membrane is seldom observed. However, when present, it (indicated with asterisk in Fig. 67), like lipid in the remainder of the cuticle is surrounded by a dense mat of filaments. Some of these filaments appear to be identical to the epicuticular filaments observed over the other gland cells and in the unmodified cuticle (compare ef (black arrows) with lt (white arrows) in Fig. 67). Favourable cross and longitudinal sections of the lipid tubules (lt) reveal one important structural difference from epicuticular filaments. The 'lipid' tubules have a very dense osmiophilic core (white arrows in Figs. 67, 68). Usually the outer walls are more intensely osmiophilic than normal epicuticular filaments but this may result from the plane of sectioning. Both filaments and tubules are approximately the same diameter.

These 'lipid' tubules approach the epicuticle in a bunch (Fig. 68). They appear to exit from the epicuticle via pores in the inner cuticulin (p in Fig. 68). The pores are structurally similar to those observed in cuticle which does not contain lipid deposits (Fig. 54) and in the spruce budworm (Fig. 35, insert). The pores in the cabbage looper cuticle are about 200Å in diameter and have a dense osmiophilic core (as observed in surface sections from a gland where 'lipid' tubules were observed within and outside the cuticle). The 'lipid' tubules outside the cuticle, that is, on the surface of the insect, have the same diameter and appearance as those approaching the pores (Figs. 55, 68). The outer cuticulin, although present in those sections of cuticle from young insects and where there is no outer layer of tubules, is not always observed when tubules are present (Figs. 54, 55, 68).

The interpretation of the spatial relationship of the various

organelles within an adult gland cell of the cabbage looper and the structure of the cuticle are presented in Fig. 70. The adult gland cell of the modified intersegmental membrane is hypertrophied and columnar in contrast to the flattened epidermal cell of the unmodified intersegmental membrane (Fig. 18). Organelles characteristic of the adult gland cell of the cabbage looper differ from those organelles of adult gland cells of the spruce budworm (Fig. 35) and the tussock moth (Fig. 43). Lipid spheres are present throughout the gland cell of the cabbage looper whereas they are only present near the base of the spruce budworm gland cell and not present in the cell of the tussock moth. Lipid spheres in the cabbage looper gland cell are usually surrounded by microbodies whereas this association is not evident in the spruce budworm gland cell where microbodies are usually found near the apical region of a cell. As in the gland cell of the tussock moth there are portions of the apical plasma membrane of the cabbage looper gland cell which lack microvilli. In contrast to the tussock moth this absence in the cabbage looper gland cell is not associated with a hyperdevelopment of smooth endoplasmic reticulum. In contrast to the gland cells of both the spruce budworm and the tussock moth, the gland cell of the cabbage looper has a distinctive basal lamina and deposits of lipid are observed in the cuticle.

Solvent Extraction of Glands

The appearance of gland cuticle after extraction with hexane or methylene chloride is similar in all three species. Changes in the endocuticle are very slight and epicuticular filaments remain unchanged. The 'uneven layer' located external to the outer cuticulin (Figs. 34, 41,

54, 55, 56) is completely absent from sections.

The lamellae of the endocuticle are less distinct in the gland cuticle of the spruce budworm and cabbage looper after extraction with chloroform/methanol. The epicuticular filaments no longer appear as tubules rather they are amorphous electron dense rods. (These observations are not peculiar to gland cuticle as they also are present in the extracted cuticle from an unmodified intersegmental membrane.) After extraction the lipid tubules are absent from the surface of the cabbage looper gland cuticle and very few are observed within them. Since the lipid tubules are often not observed within gland cuticle after routine preparation, it is difficult to assess whether they would have been present before extraction.

Thick sections of cabbage looper gland cells and cuticle show no areas in the cuticle which stain green with toluidine blue and very few unextracted lipid spheres within the cells indicating that the chloroform/methanol is indeed extracting lipids from the glandular tissue.

Modification in Vicinity of Muscle Attachment

In each of the three species at lateral edges of the intersegmental membrane forming the sex pheromone gland, the cells and cuticle are modified for muscle attachment. This is the area representing part of the posterior apophysis seen attached to the gland in whole mounts (Fig. 5). As observed in ultrastructural studies of the cabbage looper the edge of the gland cell-region is signified by the absence of lipid spheres and the disappearance of layer 2 of the basal lamina. Both of these events occur at the same cell (Fig. 71).

The structure of the cuticle in this region changes very quickly with the introduction of layers of endocuticle between the epidermal cells (cm in Fig. 72). In the introduced layers the helicoidal microfibrils of the lamellae have a different pitch from that of the remainder of the cuticle. The epidermal cells flatten within the space of two or three cells (Fig. 71). Cells modified for muscle attachment have extensions of their basal membranes forming close contact with the attached muscles. (The muscle cells shown in Fig. 28 represent muscles of Group 4 as observed in whole mounts (Fig. 5b).) The gross morphology of these cells, their ultrastructure and the ultrastructure of the overlying cuticle is characteristic of regions for muscle attachment in arthropods and fully coincides with those features reviewed and described by Neville (1975).

Localization of Catalase in Gland Cells

Within adult gland cells of all three species the microbodies exhibit a positive reaction for catalase (Figs. 73, 74, 75, 76). There was no reaction in microbodies when peroxide was omitted from the medium or when amino triazole was added to it.

Spruce budworm (Fig. 73). Spherical profiles of microbodies are usually observed and have a diameter of 0.15 µm to 0.3 µm. After reaction in the DAB medium microbodies are infrequently observed adjacent to lipid spheres. These were not noted in the routine ultrastructural investigations and are undoubtedly observed after the reaction because of the increase in electron density of their contents.

Tussock moth (Fig. 74). Both spherical and elongate profiles are observed. These have diameters from 0.05 µm to 0.2 µm and lengths from

0.75 µm to 1 µm. These microbodies arise as distensions of smooth endoplasmic reticulum as determined by the presence of a reaction product within some undistended tubules of smooth endoplasmic reticulum (broken arrow in Fig. 74).

Cabbage looper (Figs. 75, 76). Profiles of microbodies exhibiting a positive reaction for catalase are extremely numerous in gland cells of the cabbage looper (Fig. 75). Many of these microbodies are in the immediate vicinity of lipid spheres.

Elongate microbodies up to 1.5 µm in length are seen almost as frequently as spherical microbodies which have diameters ranging from 0.02 µm to 0.25 µm (Fig. 76). The smaller profiles (about 0.02 µm) represent a reaction within undistended tubules of smooth endoplasmic reticulum (broken arrows in Fig. 76). These small profiles are only observed in the vicinity of the larger profiles and lipid spheres.

The microbodies in these adult sex pheromone glands exhibit all the characteristics attributed to microperoxisomes (in mammalian lipid-producing cells) as defined by Novikoff and Novikoff (1973). These characteristics are (a) they arise as distensions of smooth tubular endoplasmic reticulum (b) they are 0.1 µm to 0.3 µm in diameter (c) they are spherical or elongate (d) they contain a moderately electron opaque matrix, lack a core and (e) they exhibit a positive DAB reaction. Therefore, the microbodies as observed in the sex pheromone glands of the spruce budworm, the tussock moth and the cabbage looper are more appropriately termed 'microperoxisomes'.

When comparing the extent of the reaction as observed in cells of the three species in this study it appears that microperoxisomes are

most closely linked to lipid metabolism in cells of the cabbage looper. In cells of both the tussock moth and the cabbage looper, reacted profiles are observed as distensions of smooth endoplasmic reticulum and in all three species are present as groups within the smooth endoplasmic reticulum. However, only in cells of the cabbage looper are many microperoxisomes intimately associated with lipid spheres.

Haemocytes and the Basal Lamina of the Cabbage Looper Gland

The basal lamina underlying gland cells of the cabbage looper is distinguishable from that underlying unmodified epidermal cells even at 36 hours before eclosion. No such structural differences were observed in the spruce budworm, tussock moth or Lobesia botrana (Lalanne-Cassou, Percy and MacDonald, 1977). Also, in the cabbage looper, haemocytes were observed near the basal lamina from 36 hours before eclosion until a few hours after eclosion. In this vicinity in the tussock moth, haemocytes were also seen but not with the same regularity.

Haemocytes. Only one type of haemocyte is observed near the basal lamina of gland cells. They have several ultrastructural characteristics which enable them to be identified as granular haemocytes (as described in the literature by Akai and Sato, 1973; Lai-Fook, 1973a; Neuwirth, 1973; Beaulaton and Monpeyssin, 1976; Raina, 1976). In the cabbage looper the granular haemocytes have a few lateral projections and a flattened surface in contact with the basement membrane while the haemocoel surface is rounded. Within the cytoplasm are many distended cisternae of rough endoplasmic reticulum, containing a fibrous material. There are also granules of variable diameter (0.2 to 1.0 μm) and density

(Figs. 48, 77). The granules contain tubules (150 - 220Å diameter) which are separated from their neighbours by fibrous extensions of about 450Å to 500Å (Figs. 78, 79, 80). Granules are sometimes near the concave, presumably maturing, face of Golgi complexes (Fig. 80) but more frequently are located near the periphery of the cells (Fig. 77). Lipid spheres are often closely associated with the granules (Fig. 80).

Another type of haemocyte is observed near the basal lamina of unmodified epidermal cells. They are roughly rounded with several pseudopodia-like extensions (Fig. 17). The following ultrastructural characteristics enable them to be identified as plasmatocytes as described in the literature. They contain rough endoplasmic reticulum, free ribosomes, a few Golgi complexes and microtubules. A few microbody-like organelles are also contained in the cytoplasm. There are no granules or lipid spheres (Fig. 17).

A rough estimate of the numbers of haemocytes associated with the basal lamina was determined from thick (1 µm) sections which encompassed both the glandular intersegmental membrane and unmodified intersegmental membrane. Tissue was obtained from 4 insects which ranged in age from EC + 1 hour to EC + 9 hours and some 10 to 15 sections were observed from each. It was found that in each section 30 to 50 granular haemocytes were adjacent to gland cells while only 3 to 10 plasmatocytes were adjacent to unmodified epidermal cells.

The basal lamina. At 36 hours before eclosion, the basal lamina underlying gland cells is clearly different from that of other epidermal cells (compare Fig. 11 with Fig. 17). There is a thin amorphous

layer (layer 1) which is also present beneath unmodified cells and a second layer (layer 2) apposing the haemocytes (Figs 48, 77, 78). Layer 2 is sometimes represented as ragged extensions of layer 1 (Figs. 48, 77). More often it is a definite layer which has a distinctly banded appearance even at very low magnifications. In older insects the width of the banded layer is fairly constant.

The banding pattern of layer 2 results from tubules (150Å in diameter) which are often in bundles and sometimes run parallel to the long axis of the cells (Figs. 44, 78). The tubules are separated from each other by fibrous extensions of about 450Å to 500Å (Fig. 79). Within layer 1, tubules are single or in small groups (Figs. 44, 79) but are reorganized as in layer 2 immediately next to the basal plasma membrane and within the basal infolds (Fig. 45). (Layer 1, when sectioned perpendicular to the long axis of the cell has the same appearance as observed in Figs. 77 and 79.)

Association of haemocytes with the basal lamina of the pheromone gland. At the time that layer 2 can be identified in the basal lamina of late pupae and early adults, granular haemocytes are particularly numerous near layer 1. They are less frequently observed in this position in older insects. Within the haemocytes next to the basal lamina granules are often situated near the periphery of the cells where they appear to be in various stages of emptying their contents into the haemocoel (Figs. 78, 79, 80). From a comparison of the dimensions and the structural appearance of tubules within the granules and in layer 2 (Fig. 79) it is suggested that the contents of the granules form layer 2 of the basal lamina (Fig. 69 (Step 1)). Thus the bundles of

tubules found forming layer 2 of the basal lamina in late pupae and young adults (Fig. 78) arise from the contents of a single granule

Granular haemocytes have also been seen between developing gland cells in the vicinity of tracheole penetration (Fig. 50). On these occasions the basal lamina beneath the tracheole/haemocyte complex consists of only the amorphous inner layer. Between the complex and the gland cells there is some evidence of the banded layer (Fig. 50).

This relationship between granules of the haemocytes and layer 2 of the basal lamina does not occur underneath unmodified epidermal cells where the basal lamina is thin and amorphous (Fig. 17). Nor does it occur near the lateral edges of the gland at the sites of muscle attachment where, underneath these modified epidermal cells, the basal lamina is also thin and amorphous (Figs. 71, 72).

Radioactive Labelling of Gland Lipids in the Cabbage Looper

In cabbage looper adults within the first hour after eclosion the gland cells contain few lipid spheres whereas by 8 hours after eclosion considerable lipid has accumulated (Figs. 22, 23). The data indicates this time interval to be one of considerable lipid synthesis. The sex pheromone of the cabbage looper is cis-7-dodecenyl acetate, therefore, C^{14}-labelled dodecanoic (lauric) acid and sodium acetate were injected into insects less than 1 hour after eclosion to determine whether any labelling could be found in lipids of the gland and if so whether one of the final stages in the biosynthesis of the pheromone is the desaturation of the corresponding acid or whether the preferred route is one utilizing acetate as a precursor. The techniques used were autoradiography and scintillation counting of extracted lipids.

Scintillation counting

Total recovery of labelled material. The following amounts of labelled material were injected into each insect: lauric acid-C_1^{14}, 350,900 dpm; sodium acetate-C_1^{14}, 788,900 dpm; sodium acetate-C_2^{14}, 644,900 dpm. Of the amount injected 166,407 dpm or 47.3% of the lauric acid-C_1^{14}; 38,177 dpm or 4.8% of the sodium acetate-C_1^{14}, and 121,417 dpm or 18.8% of the sodium acetate-C_2^{14} were incorporated into extractible compounds after 8 hours (Tables II and IV). After 3 days the percentage of recovered labelled material dropped by one-half for lauric acid-C_1^{14} and sodium acetate-C_2^{14}. This was presumably due to material lost through metabolism.

The head, thorax and wings, abdomen and gland represent respectively 56.5%, 43.1% and 0.23% of the body weight (Table III). When insects were injected with lauric acid and incubated for 8 hours a greater percentage of labelled material was recovered from the site of injection, the abdomen (85.9%, Column 4, Table II) than would be expected from an even distribution of the compound throughout the insect (each abdomen represents 43.1% of body weight). After 3 days incubation more of the labelled compounds were recovered from the head, thorax and wings (62.8%) than from the abdomen (36.8%). These results suggest that after 3 days incubation, the labelled compounds resulting from lauric acid-C_1^{14} are distributed with respect to weight in the head, thorax and wings, and abdomen. For both time intervals the percentage recovery in the gland portions remains as would be expected from a distribution with respect to weight. Thus the lauric acid is not selectively absorbed by the gland cells.

After incubation with labelled sodium acetate the percentage recovery of labelled materials from the head, thorax and wings, and abdomen remains evenly distributed (about 50%) (Column 4 Table II) as would be expected from the percentage body weight (about 50%) (Table III). The percentage of C^{14} recovered from the gland indicates that more of the labelled acetate is being extracted than is accountable by even distribution with respect to weight (i.e. 2.8 and 2.6% for sodium acetate-C_2^{14}, and 2.8 and 1.0% for sodium acetate-C_1^{14}) (Column 4, Table II) as compared with 0.23% of body weight (Table III). These results suggest that some of the labelled acetate is being incorporated into gland lipids.

When compared with the amount injected, very little labelled material was recovered from the gland (Column 1, Table IV) with 0.1% after 8 hours and 3 days when lauric acid or sodium acetate-C_1^{14} were injected. More of the sodium acetate-C_2^{14} was incorporated after 8 hours (0.5%) than the other precursors but this dropped to 0.2% after 3 days. The acetate-C_2^{14} labelled materials is presumably greater than acetate-C_1^{14} materials because some of the acetate-C_1^{14} label may be lost as carbon dioxide (Appendix 5).

Recovery of labelled compounds in lipid extract. When the incorporation of C^{14} labelled precursors into lipid (Column 2, Table IV) is compared with the total incorporated by the tissues (Column 1, Table IV) the distribution is similar for each region of the body. The percentage incorporated into gland lipid is greater for sodium acetate-C_2^{14} than for the other precursors. After 8 hours the sodium acetate-C_2^{14} incorporation is 0.4% as compared with lauric acid incorporation of 0.1%.

This can be explained by the previous comparison where it was suggested that the recovery of lauric acid is only that expected from uniform distribution within the insect.

The percentage recovery of labelled lipid from the gland (Column 3, Table IV) is essentially the same for sodium acetate-C_1^{14} (3.1%) and sodium acetate-C_1^{14} (3.0%). This is to be expected since the sodium acetate-C_1^{14} which remains after some of the label is lost as CO_2 is the same as acetate-C_2^{14} and they should be incorporated equally into lipids.

The percentage incorporation of sodium acetate-C_1^{14} (1.0%) and sodium acetate-C_2^{14} (1.7%) into lipid of the gland after 3 days decreased similarly which indicates that the material is being lost from the body at about the same rate.

Autoradiography

Reduced silver grains overlying gland cells, fat body and haemocytes in sections were no greater in number than those of the background after 24 hours exposure to the emulsion. After 6 days exposure the background had increased considerably so that it was not practical to expose the sections any longer. With the technique used 1-day-exposure is equivalent to 10 days exposure using standard techniques (Retnakaran, 1974).

The results obtained from the experiments using extracted lipid partially explain why the autoradiographic experiments were unsuccessful. The solvent used in extraction removes the sex pheromone as well as other lipid, from the gland. The weight of extracted lipid from glands of this insect has been reported as 1 mg and this contains about

500 ng of pheromone (0.05%) (Gaston et al., 1966). From the present study the maximum and minimum dpm for gland lipid from a single gland are respectively 2421 and 262 (Column 1, Table 2). The estimated maximum and minimum dpm for pheromone from a single gland would be 1.2 and 0.13 dpm. Using the technique of autoradiography this amount of radioactivity would be no higher than background especially since these figures represent dpm/gland and the technique employs 1 µm-sections of glands. This explains why no results were obtainable from these experiments. Therefore, with the precursors used, the time of injection, and the amount of labelled material available, detection of radioactivity by scintillation spectrometry was more practical.

The Defensive Gland and Its Secretion in the Red-Humped Caterpillar

Location and morphology of the gland

There are four larval instars in laboratory-reared red-humped caterpillars (Fig. 81). Although the gland is present in larvae as young as the second instar, only larvae in third and fourth instars react defensively to an external stimulus.

The gland is situated in the thorax and consists of two sacks referred to in this report as anterior and posterior glands joined by an interglandular neck. The orifice opens into a transverse invagination of the integument at the cervical margin of the prosternite (Fig. 82).

The anterior gland is pear-shaped and is about 2.2 mm long (Fig.

82). Near the orifice, the gland takes the shape of a cylindrical secretory duct. It increases in diameter posteriorly and is invaginated where it receives the interglandular neck. This entire region of the anterior defensive gland has two lateral folds extending along its length and part of the interglandular neck. Both the anterior gland and the interglandular neck have a distinctly corrugated appearance due to annular cuticular folds lining the lumen.

The posterior gland is about 4.3 mm in length (Fig. 82). When filled it extends obliquely to the right posterior dorsal margin of the metathorax and lies within the hump (arrow in Fig. 81). It has an irregular shape when empty. The end proximal to the orifice has a slight invagination where it comes in contact with the anterior gland and usually obscures the interglandular neck. The cuticular lining of the posterior gland is composed of irregularly shaped truncated papillae having intricately furrowed apices.

Musculature of the gland is restricted to the area surrounding the orifice where bilaterally paired muscles have their insertion. In his description of the musculature and tracheation, Detwiler (1922) describes a 'sub-apical lump' near the orifice. Even after repeated examination of several glands such a lump could not be found.

Chemistry of the secretion

GLC analysis of the methylene chloride extract of the anterior and posterior glands suggested the presence of a major aliphatic component in both the anterior and posterior glands (Fig. 83, A & B, peak a). This component represents at least 70% (70% posterior gland, 82% anterior gland) of the material that was detected by GLC in the neutral

fraction. There is approximately 10X as much of this material in the anterior gland as compared to the posterior gland. The retention time (RT) of this component on OV-1 was 215 sec. and on Carbowax 20M was 177 sec. The mass spectrum of the unknown compound exhibited a base peak at m/e 43, a diagnostic peak at m/e 61, and the highest upfield peak at m/e 140 (M^+-60). Peaks at m/e 43 and m/e 61 are indicative of acetates, the former representing the acetylium ion, $(CH_3CO)^+$ and the latter representing protonated acetic acid (McLafferty, 1967). It is well known that acetates do not exhibit a molecular ion (Budzikiewicz, et al., 1967) the highest upfield peak being (M^+-60) resulting in a molecular weight of 200 for the unknown compound. From this data it was concluded that the compound is n-decyl acetate.

Comparative studies using authentic n-decyl acetate confirmed the identification. The R_T of authentic n-decyl acetate on OV-1 was 217 sec. and on Carbowax 20M was 178. The mass spectra of the two compounds were identical (Fig. 84).

Ultrastructure of gland cells

Cells near the orifice. Cells of the anterior gland near the orifice are flattened with large, lobed nuclei containing several nucleoli (Fig. 85). Microvilli are sparse and often absent. The cytoplasm contains many mitochondria and disorganized rough endoplasmic reticulum. The basal lamina is noticeably thick, 5 µm, which approximates the thickness of the cuticle.

Within the soft, untanned cuticle are dense patches of mesocuticle near the tips of the cells. These patches are not continuous and there is always an arc present at the base of each cuticular trough.

Epicuticular filaments are seldom seen within the endocuticle but are located near the epicuticle.

Cells within the anterior and posterior glands. There are no ultrastructural differences between cells of the anterior and posterior glands (Fig. 86). They have an odd shape with the main portion of each cell located within internal cuticular folds. Lateral interdigitation of the cells is very complex particularly in the troughs between the folds or papillae in this vicinity, cells are often so shallow that the involutions of the basal plasma membrane and the folds of the apical membrane are very close to each other (Fig. 86). The nuclei are large and lobed. Mitochondria are numerous with an obvious separation in size of the profiles. Those located near the apical plasma membrane are often two to three times as large as those in the remainder of the cytoplasm. Rough endoplasmic reticulum predominates and along with free ribosomes is found throughout the cells (Fig. 87). The only place smooth endoplasmic reticulum is located is near the apex of the cells in the vicinity of the apical folds (Fig. 87).

The apical plasma membrane has a peculiar array of folds and depressions. In longitudinal section they appear as normal microvilli (Fig. 87). However, in cross section it is apparent that there is no regular pattern in their formation (Fig. 88). The only uniformity which exists is in the cylinders of endocuticle which invert the apical membrane. At the interior of the plasma membrane involutions, and running parallel to them, are numerous tubular membranous structures (arrows in Fig. 87 and tu in Fig. 88). These structures run the entire length of the folds, they are oval in cross section and are all about 250Å by 400Å throughout their entire length. There are several indications near bases

of folds (Fig. 87) that these tubules arise from the smooth and rough endoplasmic reticulum but tubules differ from the endoplasmic reticulum in that the dimensions of tubules are not variable.

The cuticle overlying the secretory cells is very thin (1 μm to 2 μm) and the endocuticle is very loosely laminated (Fig. 87). Tubular epicuticular filaments are not evident. However, they may be represented by fine filamentous structures in the same vicinity as expected for epicuticular filaments.

Cells within the anterior lateral longitudinal folds. These cells differ from other cells of the anterior and posterior glands in that in all samples examined they contain immense quantities of lipid (Fig. 89). Although the greater portion of the lipid is located between the nucleus and the base of the cell, it is by no means confined to this region. The lipid deposits are surrounded by mitochondria.

The cuticle overlying cells with lipid is identical to that over the other secretory cells of the anterior and posterior glands.

Cells within the interglandular neck. Cells of the interglandular neck are columnar and the nuclei large and round (Fig. 90). Microtubules, rough endoplasmic reticulum and free ribosomes are abundant. As in cells near the orifice, the microvilli when present, are very small. Near the bases of the cells are occasionally large lipid deposits. The lipid does not react very strongly with the osmium tetroxide and can be easily confused with the large electron lucent areas arising from the deeply infolded basal membrane. The basal lamina underlying these cells is very thin (approximately 0.25 μm).

The cuticle overlying the cells is 3 μm to 5 μm thick and is composed mainly of loosely laminated endocuticle (Fig. 90).

DISCUSSION

Morphology and Eversion of Sex Pheromone Glands

The surface of the sex pheromone gland of the spruce budworm has cuticular projections or 'spikes'. The projections probably increase the surface area of the cuticle but a more plausible explanation of their function is to retard the evaporation of the pheromone from the surface of the gland. This latter suggestion is supported when it is noted that 'spikes' also occur on the cuticle of the cabbage looper gland (also noted by Jefferson et al., 1968) and that, in both species, once the gland is everted it remains so during the calling period (Shorey, 1964; Sanders, 1969). Similar structures ('spikes') are observed on the surface of the pheromone gland of the silkmoth, Bombyx mori, and its calling behaviour resembles the cabbage looper and the spruce budworm (Waku and Sumimoto, 1969). On the other hand, 'spikes' are not observed on the surface of the tussock moth gland and in contrast to the above species, is constantly protruding and retracting the gland when calling (Percy et al., 1971). Therefore, it is suggested that when the surface of a pheromone gland exhibits 'spikes' and the gland is constantly everted during calling, the main function of the cuticular projections is to retard the evaporation of the pheromone. A similar function has been proposed for cuticular projections, ("mushroom-shaped bodies") located on the evaporative surface of the defensive gland in the bug, Nezara viridula (Remold, 1963).

Eversion of the sex pheromone gland is controlled in the spruce

budworm by five groups of muscles (2, 2p, 3, 3p, 5), in the cabbage looper by four groups (2, 2p, 3p, 5) and in the tussock moth by three groups (2p, 3p, 5). The last species is the only one which does not completely evert the gland and one would expect fewer muscles to be involved in its eversion. Inversion of the gland is controlled by three groups of muscles which occur in all three species. In the literature there is a single report of musculature in the terminal abdominal segments of a moth as related to the retraction and protraction of a pheromone gland. The insect studied was the cabbage looper (Jefferson et al., 1968).

The gland of the cabbage looper is protracted by two sets (= groups) of longitudinal muscles (Jefferson et al., 1968). One set "has its origin on the posterior margin of the seventh tergum and is inserted on the anterior end of the apophyses anteriors and the other set originates on the posterior margin of the eighth tergum and is inserted on the anterior end of the apophyses posteriors." These muscles can be related to those of the present study as follows: the first set represents Group 2 while the second set represents Group 2p. However, Jefferson et al. (loc. cit.) do not mention the lateral muscles (Groups 3 and 3p) which, when functioning to compress the abdomen, would presumably cause protraction (eversion) of the pheromone gland.

Retraction of the gland of the cabbage looper is by means of "longitudinal muscles which have their origin in the seventh segment and insert on the valves, the common oviduct, the apophyses anteriors and the apophyses posteriors" (Jefferson et al., 1968). These muscles can be related to those of the present study as follows: those which

insert on the valves (= ovipositors) actually insert on the lateral edges of the gland as Group 4, those which insert on the anterior apophyses are Group 1, those which insert on the posterior apophyses are Group 1p.

Finally, although it has been shown that there are more groups of muscles associated with sex pheromone glands than has previously been reported, their presence does not exclude the possibility that the eversion and inversion of the pheromone gland may be influenced by the presence of, or factors from, other tissues.

Haemocytes and the Basal Lamina

Haemocytes of insects have been the subject of several recent ultrastructural studies. There are four or five types of haemocytes which are common to most of the insect larvae of the order Lepidoptera. These are prohaemocytes (or stem cells), plasmatocytes, granular haemocytes, oenocytoids and sphaerule cells (Akai and Sato, 1973; Lai-Fook, 1973a; Neuwirth, 1973; Beaulaton and Monpeyssin, 1976; Raina, 1976). Intermediates between the cell types have also been observed (Lai-Fook, 1973a).

In the adult cabbage looper the structural characteristics of the haemocytes underlying unmodified epidermal cells correspond to those ascribed to plasmatocytes (Lai-Fook, 1973a; Beaulaton and Monpeyssin, 1976). In contrast, those haemocytes underlying the gland cells contain structures characteristic of granular haemocytes, i.e., secretory granules and lipid spheres (Akai and Sato, 1973; Lai-Fook, 1973a; Neuwirth, 1973; Beaulaton and Monpeyssin, 1976; Raina, 1976). The present study indicates that one function of these secretory granules

may be the formation of at least part of a specialized basal lamina. In this respect the granular haemocytes also fit into a group termed 'trephocytes', functionally described as secretory or storage haemocytes in insects (Crossley, 1975).

Wigglesworth (1959) first suggested that adipohaemocytes[2] in Rhodnius prolixus are secretory and participate in the formation of basement membranes (= basal laminae) of epidermal cells. This hypothesis was later supported by a study of the tunica propria (= basal lamina) surrounding the prothoracic gland of Antheraea pernyi (Beaulaton, 1968). Here a close association between granular haemocytes and the tunica propria was observed as the latter increased in thickness. In addition cytochemical characteristics common to the granules and the tunica propria were observed. In 1973, Wigglesworth reported a re-examination of haemocyte involvement in basal lamina formation of epidermal cells in fourth instar Rhodnius prolixus after feeding. Within plasmatocytes, underlying the basal lamina, the contents of granules are tubular, similar to granules in granular haemocytes of the cabbage looper. However, in contrast to observations in the present study the contents of granules from plasmatocytes lose their tubular appearance immediately after release. This secretion participates in the formation of the basal lamina, the latter remaining amorphous ("fibrous") although it is noticeably thickened as a result

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[2] In Heteroptera adipohaemocytes = plasmatocytes (Wigglesworth, 1973). In Lepidoptera these plasmatocytes are structurally comparable to granular haemoctyes (Beaulaton and Monpeyssin, 1976).

of material contributed by the plasmatocytes. On the other hand, Lai-Fook (1970) studying wound repair in Rhodnius prolixus, found no evidence to suggest that plasmatocytes played a significant role in basement membrane formation. Studies concerning the involvement of haemocytes in basal lamina formation of various other tissues has been recently reviewed by Crossley (1975).

The basal lamina underlying sex pheromone gland cells of the cabbage looper differs in structure and presumably, therefore, in function, from that underlying unmodified epidermal cells. The function of the unusual bilayered basal lamina is not easily determined. Haemocytes assist in its formation in very young adults but the bilayered structure remains throughout the adult stage (at least until four days old). Pheromone extract from glands of young adults (emergence to 1 day old) has about 1/100 of the biological activity of that from two to four-day-old adults (Shorey et al., 1968). Therefore, it is possible that the granules, and, in turn, the basal lamina contain some substance which may be used in the formation of the sex pheromone. The chemical structure of the sex pheromone is cis-7-dodecenyl acetate (Berger, 1966). With present knowledge it is difficult to perceive how this compound is related to compounds usually found associated with granules in haemocytes of other insects: mucopolysaccharides or muco- or glycoproteins in A. pernyi (Beaulaton, 1968; Beaulaton and Monpeyssin, 1976); acid mucosubstances in Calpodes ethlius (Lai-Fook, 1973a) and sulphated periodate-reactive sialomucin with some glycoprotein or neutral polysaccharide in Locusta migratoria (Costin, 1975). Two recent studies have illustrated the presence of a lipid or lipid complex associated with granules and/or basal lamina. In Rhodnius

prolixus lipids, proteins and carbohydrates have been detected both in granules of haemocytes and the basal lamina underlying epidermal cells (Wigglesworth, 1973). In Galleria mellonella a lipoprotein has been identified as one of the constituents of the basal lamina of the fat body (Dutkowski, 1977). At present, the constituents of basal laminae in the cabbage looper are unknown.

Near the basal lamina of the cabbage looper within the haemocytes there is a close association between lipid spheres and granules. The lipid is, in all likelihood, released with the contents of the granules, either separately or as a complex. Since the structural components of the granules are evident both in basal infolds and between adjacent cells, and coated vesicles are prevalent in both positions, it is possible that one or both components are taken up by gland cells. Such pre-formed complex lipids may then participate in biosynthesis of pheromone. Therefore, this hypothesis is included as a preliminary stage in the interpretation of the formation and transport of lipid within gland cells (Fig. 69 (Step 1)).

Gland Cell Ultrastructure and Pheromone Synthesis

Adult females of the spruce budworm, the whitemarked tussock moth and the cabbage looper are most attractive to males for limited time periods after eclosion. Female tussock moths are most attractive 12 to 24 hours post eclosion (Percy et al., 1971; Grant, 1975) while female spruce budworm and cabbage looper are most attractive from 2 to 4 days post eclosion (Shorey et al., 1968; Sanders and Lucuik, 1972a). From such observations it is surmised that the sex pheromone

gland is producing the maximum amount of pheromone at these times. The glands of the female cabbage loopers at 2 to 4 days of age actually contain considerably more sex pheromone than do glands from either younger or older moths (Gaston et al., 1966). The pheromone for each of the species is a lipid. (For the spruce budworm it is trans-11-tetradecenal and cis-11-tetradecenal (Weatherston et al., 1971; Sanders and Weatherston, 1976). For the cabbage looper it is cis-7-dodecen-1-ol acetate (Berger, 1966). For the tussock moth it contains cis-6-heneicosen-11-one (Grant and Frech, 1976; Grant, 1977).)

The structure of the gland cells in all three species is consistent with the hypothesis that these cells are indeed involved in lipid production and thus it can be deduced that they are involved in pheromone production.

Lipid spheres. The accumulation of lipid spheres within the cytoplasm of gland cells from the spruce budworm and the cabbage looper is the most obvious illustration of the function of the cells. This feature is characteristic of most pheromone gland cells from female moths and can be detected even in paraffin embedded sections where the lipid spheres are referred to as vacuoles (Dickens, 1936; Steinbrecht, 1964a; Goerge, 1965: Barnes et al., 1966; Jefferson et al., 1966, 1968; El-Sawaf et al., 1968; Roelofs and Feng, 1968; Weatherston and Percy, 1968; Jefferson and Rubin, 1970; Stanic et al., 1970; Percy and Weatherston, 1971a; Percy et al., 1971; Thibout, 1972). To date there have been very few ultrastructural studies of pheromone gland cells but these also report the presence of lipid spheres (Steinbrecht, 1964a: Waku and Sumimoto, 1969; Smithwick, 1970; White et al., 1973: Feng and Roelofs, 1977). From his observations of

the gland cells in Bombyx mori, Steinbrecht (1964a) concluded that although the lipid spheres contained unsaturated neutral lipids (which include the sex pheromone, bombykol) it was unlikely that the lipids represent the final secretion. This is confirmed by the observations of the spruce budworm gland cells where the size and distribution of lipid spheres does not alter with the age of the adult insect even after the insect ceases to be maximally attractive to the males (i.e., after EC + 4 days). The hypothesis is only partially confirmed by gland cells of the cabbage looper. In support of the hypothesis the lipid in the cells does not decrease with the age of the insect. However, the size and distribution of the lipid spheres correlates very well to the age of the insect and the pheromone content of the gland. Lipid also moves into the cuticle which indicates that some pheromone is present. In contrast to the results obtained from gland cell ultrastructure of these other species, no lipid spheres are observed in pheromone gland cells of the tussock moth.

Smooth endoplasmic reticulum. Gland cells of the tussock moth as well as those of the spruce budworm and the cabbage looper, contain smooth endoplasmic reticulum which appears in the cells immediately before each gland releases its pheromone. Extensive development of smooth endoplasmic reticulum indicates lipid synthesis as illustrated by observations of epidermal gland cells in other insects where the secretion is at least partly composed of lipids. These studies include the columnar cells, but not the glandular cells, of the sternal glands of the termites Trinervitermes geminatus (Quennedey, 1972) Kalotermes flavicollis (Quennedey, 1971) and Zootermopsis nevadensis (Stuart and Satir, 1968); the cells of Gilson's gland in larvae of Phryganea varia

(Quennedey, 1969); certain cells of the defensive gland of Eleodes longicollis (Eisner, et al., 1964); the wax gland of larvae of Calpodes ethlius (Locke, 1969b) and the lateral scent gland of Nezara viridula (Filshie and Waterhouse, 1968). Smooth endoplasmic reticulum appears at about the time of eclosion in the sex pheromone gland cells of Bombyx mori (Steinbrecht, 1964a), Plodia interpunctella (Smithwick, 1970) and Lobesia botrana (Lalanne-Cassou et al., 1977).

Perhaps the most convincing evidence relating the appearance of smooth endoplasmic reticulum to lipid synthesis is deduced from studies of mammalian cells. In certain testicular cells conversion of the cytoplasm from one which consists mainly of rough endoplasmic reticulum to one which is predominantly smooth endoplasmic reticulum is coincident with the first appearance of steroids synthesized by these cells (Black, 1972). Furthermore the smooth endoplasmic reticulum contains major steroidogenic enzymes (Inano et al., 1969). In absorptive cells of the mammalian intestine, the formation of smooth endoplasmic reticulum corresponds to the absorption and resynthesis of triglycerides (Cardell et al., 1967).

Although the gland cells from the three species under study here are similar in the respect that they all contain smooth endoplasmic reticulum, the position it occupies in each of them differs. In the spruce budworm it is tubular and located primarily between the nucleus and the apical plasma membrane; in the tussock moth it is both cisternal and tubular and when present is located throughout the cell; in the cabbage looper it is mainly tubular and is located primarily near the lipid spheres. Some smooth tubular endoplasmic reticulum is also observed near the lipid spheres in the spruce budworm. In the other

studies of insect epidermal cells mentioned above, the position of the smooth endoplasmic reticulum relative to the other organelles was usually not mentioned. However, in the sex pheromone gland cells of Lobesia botrana the smooth endoplasmic reticulum is present throughout the cells (Lalanne-Cassou et al., 1977).

Microperoxisomes. Another comparable feature of adult gland cells in the three species under study is the presence of microbodies. These microbodies fit the dimensions and have the morphological characteristics of those observed in several mammalian cells (Hruban et al., 1972) and those of larval oenocytes (Locke, 1969b). The microbodies of the gland cells exhibit a positive reaction for catalase as demonstrated in the alkaline DAB technique of Novikoff and Goldfischer (1969) and modified by Novikoff et al. (1972) and are, therefore, more correctly termed 'microperoxisomes' (Novikoff and Novikoff, 1973; Novikoff, A.B. et al., 1973a; Novikoff, P.M. et al., 1973).

In sex pheromone gland cells the microperoxisomes occur in large numbers during the time when the females are most attractive to males in all three species, therefore, they occur when the (lipid) sex pheromones are being produced. More specifically, in cells from the cabbage looper, microperoxisomes are most prevalent during the time when the largest amount of pheromone is produced by the gland. This also coincides with the time that most lipid spheres are seen in the cytoplasm. Therefore, it appears that microperoxisomes might be involved in lipid metabolism in these cells. This is supported from many studies of mammalian tissues where their role in lipid metabolism from lipid-metabolizing tissue appears to be universal although they also occur in other types of tissue (Hruban et al., 1972). In lipid-metabolizing

tissue they have been implicated in steroid metabolism (Reddy and Svoboda, 1972; Black and Bogart, 1973; Reddy, 1973), catabolism of absorbed lipids in gut cells (Novikoff and Novikoff, 1972; Novikoff et al., 1972), degradation of lipids in lipofuscin granules of human hepatocytes (Novikoff, A.B. et al., 1973b). An interesting relationship exists between the appearance and disappearance of microperoxisomes in hepatocytes of rats fed hypolipemic drugs which cause a fatty liver (Goldfischer et al., 1971; Reddy and Svoboda, 1972; Reddy, 1973). When two of these specific drugs are fed separately to rats a fatty liver occurs, that is, the hepatocytes contain a proliferation of smooth endoplasmic reticulum and microperoxisomes in association with lipid spheres. If, after the reaction is initiated by one drug, a second drug is fed to the rat, the whole reaction is reversed. These ultrastructural observations have been confirmed by biochemical observations of serum and liver triglycerides (Novikoff et al., 1974; Novikoff and Edelstein, 1977).

The relationship of the enzyme, catalase, to lipid synthesis is not clear either from the literature or from its appearance in sex pheromone glands. Catalase normally oxidizes endogenous H_2O_2 with H_2O_2 to form water but under the conditions outlined for this reaction it acts peroxidatically to oxidize DAB (Novikoff et al., 1973). Within microperoxisomes it has been suggested that the catalase detoxifies the H_2O_2 produced by various oxidases which have also been located in some microperoxisomes. Several of these oxidases, including fatty acid oxidases, have been identified, the particular one varying with the tissue under investigation. Other enzymes also associated with lipid

metabolism have been located in microperoxisomes of some tissues but, as with the oxidases, their direct functional significance is unknown (McGroarty and Tolbert, 1973). In larval fat body of Calpodes ethlius microperoxisomes have been shown to be related to the production of the non-lipid excretory products of an insect. These contain urate oxidase and catalase and are morphologically similar to microperoxisomes although they contain a core similar to peroxisomes (Locke and McMahon, 1971).

Another function of catalase as related to lipid metabolism may occur in those microbodies where a peroxide-producing oxidase cannot be demonstrated. Catalase itself, under the right conditions, can act as a powerful lipid peroxidation catalyst, substituting for lipooxidases in animal tissues where these latter enzymes are not found (Stumpf, 1970; Black and Bogart, 1973).

With respect to cells of the sex pheromone glands no attempt can be made at present to identify enzymes other than catalase in the microperoxisomes, or to relate catalase itself to the biosynthesis of the pheromones. Hypotheses can be formulated and tested only with certainty after some knowledge is obtained concerning pathways involved in the biosynthesis of pheromones.

Golgi complexes. These organelles appear to be intimately involved in the accumulation of lipid as exemplified by their proximity to lipid spheres, and developing lipid spheres, in gland cells of the cabbage looper. Golgi complexes have also been observed near lipid spheres in gland cells of the spruce budworm and near groups of microperoxisomes in gland cells of the tussock moth. The function of the Golgi complex (as reviewed by Morré et al., 1971) includes product transformations,

in addition to its more general role in membrane transformations. The product transformation, with reference to lipid metabolism, includes an intricate involvement in the synthesis and transport of (lipoprotein) secretory particles in hepatocytes (Morré et al., 1971; Novikoff et al., 1974). Therefore, in sex pheromone gland cells, where the final secretory product is a lipid, the Golgi complex may effect structural changes in precursors of the secretory product or to enzymes involved in its pathway. Answers to such speculations may be obtained through a thorough structural study of Golgi complexes in sex pheromone gland cells and cytochemical analysis of contents and/or secretion, particularly in cabbage looper gland cells.

Microvilli. The microvilli of all three adult gland cells are similar in that they contain a tubule of smooth endoplasmic reticulum. Penetration of microvilli by smooth endoplasmic reticulum has been reported in cells of the epidermal glands of Phryganea varia (Quennedey, 1969), Kalotermes flavicollis (Quennedey, 1971), Calpodes ethlius (Locke, 1969b) and Zootermopsis nevadensis (Stuart and Satir, 1968), and in cells of the tubular-arm of the osmeterium of Papilio larvae (Crossley and Waterhouse, 1969) and in Verson's glands of Calpodes larvae (Lai-Fook, 1973b). Although not reported as such, the appearance of the microvilli in the electron micrographs is very similar in cell 1 of the defensive gland of Eleodes longicollis (Eisner et al., 1964) and the hair pencil gland of Danaus gilippus berenice (Pliske and Salpeter, 1971). The microvilli of the columnar cells of Trinervitermes geminatus consist of two types, narrow with little internal organization and wider, less numerous ones. Each of the latter is penetrated by smooth endoplasmic reticulum and microtubules, and each extends much

further into the cuticle than the smaller ones (Quennedey, 1972). It is noteworthy that these cells, in which the structure of the microvilli is basically similar, all secrete lipid-soluble substances. On the other hand, the folds of the apical membrane of the sex pheromone glands of the following species have no internal organization; Bombyx mori (Steinbrecht, 1964a), Lobesia botrana (Lalanne-Cassou et al., 1977) and Agyrotaenia velutinana (Feng and Roelofs, 1977). The difference in structure of the apical projections of gland cells in these last mentioned insects may indicate a difference in mode of transmission to the cuticle of pheromone, pheromone precursors or enzymes involved in pheromone biosynthesis.

From the present study the indications are that the presence of the microvilli is transitory and may be related to the function of the cell at that particular time. For example, microvilli were sometimes not observed on the apical membrane of gland cells in the tussock moth. Also in gland cells of the cabbage looper (but not in the spruce budworm) the lipid spheres actually appeared to be extruding the portion of the apical plasma membrane originally located between microvilli. In this process, the cell membrane forming the surface of the lipid spheres would most certainly have originated from adjacent microvilli. A somewhat analogous situation occurs in vertebrate gastric parietal cells which secrete hydrochloric acid and which also exhibit microvilli containing a tubule of smooth endoplasmic reticulum. In these cells well-developed microvilli and a corresponding small amount of smooth endoplasmic reticulum are indicative of stimulated HCl secretion. The reverse occurs in cells where HCl secretion is inhibited (Ito and Schofield, 1974). Modifications of the microvilli of the cabbage looper gland cells

are certainly seen when they contain lipid spheres. In tussock moth gland cells, extensive development of either rough or smooth endoplasmic reticulum is often accompanied by few microvilli. This hypothesis concerning the transitory nature of the microvilli is also supported by an observation on Calpodes ethlius larvae where, during epicuticle formation, the plasma membrane passes from microvillate to smooth and back to microvillate again (Locke, 1969a).

Gland Cuticle and Pheromone Storage and Release

The structural characteristics of the cuticle overlying gland cells can be compared to those observed in other studies of insect cuticle. The filamentous structure, observed particularly well in spruce budworm cuticle, originates near the microvilli and terminates very close to them. It is unlikely that this structure represents the pore-canal filament seen in tanned and untanned cuticle as in these studies the filament traverses almost the entire cuticle (Locke, 1961; Quennedey, 1971, 1972). In gland cuticle from the relationship of its termination to the beginning of the tubular appearance of the epicuticular filaments, it is more likely that this filamentous structure is involved in the synthesis or formation of the filaments. A somewhat analogous situation is described in larval antennal pore canals of Cternica destructor where a single dense filament (albeit with a different origin) appears to branch into a series of fine filaments (Zacharuk, 1972). In Lobesia botrana however, a filamentous structure is not associated with epicuticular filaments originating near the plasma membrane between the apical folds (Lalanne-Cassou et al., 1977).

The epicuticular filaments consistently terminate near the outer

surface of the cuticulin, at an oval depression and is not peculiar to the cuticle overlying gland cells. This mode of termination was also observed in Lobesia botrana (Lalanne-Cassou et al., 1977). In other insects where the filaments penetrate the dense epicuticle in groups, there is also some indication that a depression free of epicuticular filaments is present (Filshie, 1970b; Quennedey, 1971, 1972; Steinbrecht, 1964a). The structural appearance of the base of the oval depression (particularly in the tussock moth) may be similar to the 'pore plate', a sieve-like region located at about the same position in wax canals of Blaberus trapezoideus. The wax canals are similar in appearance to the epicuticular filaments but differ in size and terminate singly in the cuticulin (Brück and Stockem, 1972a). In Blaberus trapezoideus and in Periplaneta americana, it appears that the tubular nature of the filaments is real, or at least that the clear core contains water. A solution of lanthanum hydroxide applied to the surface of the cuticle will penetrate the filaments for some distance (Brück and Stockem, 1972b).

While the structural characteristics are similar in cuticle overlying the glands of the three insects in the present study, only the gland cuticle of the cabbage looper and the tussock moth are very different from unmodified cuticle. The modifications in cuticle of the tussock moth may only occur in order to accommodate more gland cells. However, the modifications of the cuticle of the cabbage looper structurally illustrate its function, i.e. transport of lipid. As far as can be determined this is the first report of deposits of lipid taking this form within the cuticle. The two forms of filaments seen within the cuticle are not unique to the cabbage looper gland. Gilson's gland in larvae of Trichoptera also secretes lipid compounds. Two forms

of filaments have been observed within the cuticle overlying the gland cells, one of which contains a core (Quennedey, 1969). In addition, these filaments have been observed passing through the cuticulin similar to that observed in the cabbage looper.

Assuming that the lipid stored within the cuticle represents, or includes, the pheromone of the cabbage looper then several explanations may be given for its appearance there and not in the cuticle of the other two species. The structural differences may thus result from one or more of the following:

(a) The gland cells of the cabbage looper produce more pheromone than the other two species. This may be true if the amount of lipid observable within cells can be equated with the amount of pheromone produced.

(b) The cabbage looper may be more parsimonious in its use of pheromone than the other two species. At present only the release rate is known only for the cabbage looper (about 10 ng/minute) (Sower et al., 1972).

(c) Only in the cabbage looper is the pheromone, or its immediate precursors, present in the cuticle as a free lipid. In the other insects it may exist as lipoproteins or phospholipids possibly bound as structural components of the cuticle (e.g. epicuticular filaments) which would require an enzymatic reaction to release the pheromone. In support of this suggestion, when the glands were extracted with lipid solvents the epicuticular filaments were either structurally unaltered or slightly altered but not removed. In addition there is a little evidence from chemical and behavioural studies of the spruce budworm to indicate that at least one of the final reactions takes

place in the cuticle. The pheromone blend, cis and trans-11-tetradecenal can only be isolated from cut tip extracts infrequently and in small amounts (Weatherston et al., 1971). However, trans-11-tetradecenol, a known inhibitor of male response to the pheromone can always be isolated from abdominal tips (Sanders and Luciuk, 1972b; Weatherston and MacLean, 1974). Since there would be no advantage to the female releasing such a compound and since, if the conversion took place far from the cuticle surface, the pheromone would readily be detected in extracts, then the conversion to the aldehyde probably occurs near the outer cuticular surface of the gland.

Some storage of pheromone occurs on the outer surface of the gland in all three species. After solvent extraction of glands the outer 'uneven layer', as observed with the electron microscope, disappears entirely. The solvents used are the lipid solvents, hexane and methylene chloride, which are also used in the extraction of pheromones. This outer layer of insect cuticle, the so-called 'wax layer' is found on the surface of all insects (Neville, 1975). The 'uneven layer' is extracted from gland cuticle using lipid solvents, therefore, it is safe to assume that this layer represents the 'wax layer' which in a pheromone gland cuticle consists, at least in part, of the sex pheromone.

Pheromone Biosynthesis

The ultrastructure of cabbage looper gland cells illustrates the closest relationship between the appearance of organelles indicating lipid synthesis and the age of attractiveness of the adult female. The

attractiveness of the female is equated with the release of its pheromone, cis-7-dodecenyl acetate (Sower et al., 1971, 1972).

Biosynthesis of aliphatic acids and esters from 2-carbon-units has been well documented in the literature both for plants and mammals but not for insects (Stumpf, 1970; Wakil, 1970; Bressler, 1970; Synder and Malone, 1970; Spener et al., 1969; Thompson and Barlow, 1972). The pathways and interconversions involved are illustrated in Appendix 5.

From these pathways it is evident that there are at least three methods of obtaining the required product. In route 1, the usual method of fatty acid biosynthesis, acetyl CoA is the starting compound to which malonyl CoA is added. Desaturation can occur either en route or after the required chain length is attained. In route 2, the starting compound is a fatty acid having a length shorter than or equivalent to the required product. Desaturation occurs as in route 1 but is of necessity much closer to the finished product. In route 3, other compounds provide the starting fatty acids. These compounds may be triglycerides, phospholipids, glycolipids or lipoproteins. The fatty acids may be elongated, shortened and/or desaturated before they enter.

Although all of these pathways commonly occur in plants and mammals it appears that in at least four species of insects, the only product of de novo synthesis of fatty acids (Route 1) is hexadecanoic (palmitic) acid (Thompson and Barlow, 1972). There has been only one study concerning the biosynthesis of sex pheromones in moths. This was carried out on the silkmoth, Bombyx mori, where the late pupal stages were injected with palmitic acid-C_1^{14}. Labelled pheromone, trans-10-cis-12

hexadecadien-1-ol was recovered from the insect thus indicating that route 2 occurs in this insect (Inoue and Hamamura, 1972). More specifically desaturation occurs as one of the final steps in the biosynthesis of this pheromone.

Results from the biosynthetic experiments in the present study show that the final stages in the biosynthesis of the cabbage looper pheromone are not the desaturation and reduction of a 12C acid and the resultant acetylation of the corresponding alcohol. The percentage of lipids extracted from the gland after 8 hour incubation with lauric acid-C_1^{14}-(0.2%) is much lower than that for sodium acetate C_1^{14} (3.1%) or sodium acetate C_2^{14} (3.0%) (Column 3, Table 4). If one of the final stages in the synthesis was the desaturation of lauric acid then one would expect a higher percentage recovery. The results thus indicate that desaturation of fatty acids of 12C length is more difficult to realize than is the insertion of double bonds during synthesis. This coincides with Thompson and Barlow (1972) who stated that in their studies "no evidence was obtained to indicate that saturates and unsaturates are formed by completely independent mechanisms." On the other hand, Inoue and Hamamura (1972) found that in the silkmoth injected palmitic acid is readily converted to its pheromone, trans-10-cis-12 hexadecadien-1-ol. The difference may lie in the fact that the pheromone of the silkmoth is a 16C molecule which can be synthesized de novo (Thompson and Barlow, 1972).

On the other hand, there is some incorporation of labelled acetate into extracted lipids. It is possible to formulate an hypothesis concerning the relationship of the acetate to pheromone biosynthesis by combining the known methods of aliphatic ester biosynthesis with the

results of the present experiments while also remembering that the lipid extract does not exculsively contain the pheromone.

Acetate could participate in the formation of the pheromone of the cabbage looper, cis-7-dodecenyl acetate, by combining with longer pre-existing fatty acids. These are subsequently shortened to the required length with the desaturation having occurred during the synthesis of the original molecule. If the acetate is combined with a molecule shorter than the pheromone, the synthesis continues until its length is such that it can be shortened. There is no evidence to suggest that these longer molecules remain free as they may just as easily form part of larger lipid-containing molecules, such as phospholipids, triglycerides or lipoproteins.

Larger lipid-containing molecules could originate outside the cell. The function of the gland cells in this case would be the catabolism of these compounds with the end product being the pheromone. Structural representation of these interconversions within cells would probably be analogous to lipid biosynthesis from acetyl CoA. Thus although ultrastructural observations reveal that the time interval considered in the present study is one of maximum lipid synthesis, they may only represent the time of maximum lipid conversion with the actual precursors having been taken up by the cells much earlier during the pupal stage of the cabbage looper females. Furthermore, it is possible that these precursors originate from the granular haemocytes. During this time, as discussed previously, (p.), there appears to be an intricate relationship between the haemocytes and gland cells which occurs via the basal lamina of the gland cells.

Aliphatic Ester as Component of Defensive Secretion

The major aliphatic component of the defensive secretion of the red-humped caterpillar was identified as n-decyl acetate. This is the first report of n-decyl acetate in the defensive secretion of a notodontid larva. It has previously been reported, along with other acetates, in Dufour's gland of several species of slavemaking ants where it functions as an alarm pheromone (Regnier and Wilson, 1971). Various aliphatic esters, excluding n-decyl acetate, have also been identified in, and their functions demonstrated from, defensive secretions in modified mandibular glands of larvae in the family Cossidae (Trave et al., 1960; Trave et al., 1960; Trave et al., 1966; Marchesini et al., 1969).

The n-decyl acetate is an accessory component in the defensive secretion of the red-humped caterpillar, the major component being formic acid (Detwiler, 1922). Defensive secretions which consist of aliphatic and hydrophilic compounds are not unusual. The secretion from larvae of Heterocampa manteo, another notodontid, contains formic acid and acyclic ketones (Eisner et al., 1972) and the secretion from the carabid beetles, Helluomorphoides ferrugineus and H. latitarsus contains formic acid and nonyl acetate (Eisner et al., 1968). In these instances it has been shown that the lipophilic compounds act as spreading agents and aid penetration of the hydrophilic formic acid through the cuticle of an attacking arthropod. In addition, these authors suggest that the ketones have a defensive function, since by themselves they caused a defence reaction in cockroaches and spiders. Therefore, n-decyl acetate, the major aliphatic component of the defensive secretion from the red-humped caterpillar probably has a similar function.

n-Decyl acetate is a derivative of a fatty acid as are the sex pheromones of the three adult moths included in this study. The pheromone of the cabbage looper, cis-7-dodecenyl acetate, most closely resembles n-decyl acetate in chemical composition. Both are esters of fatty acids having carbon chains of similar length. The main difference is in the degree of unsaturation. However, this feature does not distinguish sex pheromones from allomones as 11-dodecenyl acetate is included in the defensive secretion of Zeuzara pyrina (Cossidae) (Marchesini et al., 1969). Therefore, it is evident that airborne semiochemicals very similar in structure are produced in the order Lepidoptera by both larvae and adults. When emanating from larvae the compounds are interspecific and related to defense, when emanating from adults they are intraspecific and related to mating.

Gland Structure as Related to Defensive Secretion

All cells of the defensive gland in the red-humped caterpillar differ considerably from cells of defensive glands in most other arthropods. Usually, cells from this type of gland contain cuticular ductules (Forsyth, 1970, 1972; Filshie and Waterhouse, 1968; Happ and Happ, 1973; Eisner et al., 1964; Percy and Weatherston, 1971b; Weatherston and Percy, 1969; and others). These arthropods secrete a range of compounds (Eisner et al., 1964; Forsyth, 1970; Happ and Happ, 1973) including dec-2-enyl acetate in Nezara viridula (Filshie and Waterhouse, 1968) and several lipoidal acetates in Blaniulus guttulatus (Weatherston et al., 1971). Also, in formicine ants where the poison gland secretes formic acid, the gland cells are also associated with a cuticular duct (Wilson and Regnier, 1971; Hermann and Blum, 1967). In addition, early

morphological and histological descriptions of the defensive gland of the red-humped caterpillar illustrated fine canals leading from the cells to the cuticle (Detwiler, 1922). He suggested that the canals were "outlets for fluids secreted by the overlying cells." These 'canals' (= ductules) were not seen in the present study either with the light microscope or electron microscope nor were ductules observed in cells of the defensive gland (osmeterium) in larvae of the butterfly, Papilio (Crossley and Waterhouse, 1969). Moreover, the description of cells in the defensive glands of other notodontid larvae, Cerura vinula and Notodonta anceps (Hintze, 1969)contains no report of cuticular ductules. Generally, it is thought that the presence of these ductules allows the cells to be spatially separated from the toxic components that they produce. It appears, therefore, that at least in the defensive glands of those lepidopteran larvae studied to date, a mechanism other than a 'ductule mechanism' is operative.

The n-decyl acetate in the defensive secretion of the red-humped caterpillar seems to originate from the anterior gland of the defensive gland complex. In support of this statement the anterior gland contains considerably more n-decyl acetate than does the posterior gland. Since the glands were removed from the insects by the duct leading to the orifice the small amount of n-decyl acetate in the posterior gland could have occurred by contamination through leakage from the anterior gland. The anterior gland is much smaller than the posterior gland. Consequently the total volume of secretion that the anterior gland could contain is much smaller than the posterior gland. Therefore, it is unlikely that the anterior gland would selectively take up the ester from the posterior gland for future use. The

defensive gland complex of Heterocampa manteo is similar in appearance to that of the red-humped caterpillar and its defensive secretion also contains accessory aliphatic compounds (Eisner et al., 1972). However, these authors used entire glands in their analyses. Therefore, it is not known whether the aliphatic compounds are located in both parts of the gland. There have been no other studies of these glands reported to which the anterior and posterior glands can be compared with respect to their contribution to the defensive secretion.

Other than the anterior lateral longitudinal folds, there are no structural modifications of the anterior gland which distinguish it from the posterior gland. The cells of the lateral longitudinal folds always contain lipid deposits and are always distinguishable from the remaining cells of the anterior and posterior glands where lipid deposits are not found. Therefore, it is possible that the cells of the lateral longitudinal folds function mainly in the production of the lipophilic n-decyl acetate or its transfer from the haemolymph. Lipid deposits are also infrequently observed within cells of the inter-glandular neck. However, in view of their location within the gland complex they probably function to maintain the form and elasticity of this region.

The molecular similarity between the cabbage looper sex pheromone and the n-decyl acetate in the defensive secretion of the red-humped caterpillar is not reflected in the ultrastructure of the gland cells. In the red-humped caterpillar the only cells with large deposits of lipid are those of the anterior lateral longitudinal folds. These deposits differ from those of pheromone gland cells in that the former are not organized into spheres. However, the apical membrane of

defensive gland cells in both anterior and posterior gland cells may exhibit some similarity to that of pheromone gland cells. The apical membrane of defensive gland cells is characterized by a complex folding instead of microvilli. However, this folding is associated with well-organized tubules which may represent tubules of smooth endoplasmic reticulum. In this respect these folds are similar to the microvilli of pheromone gland cells. As in gland cells of the spruce budworm, there seems to be no variation in this association in contrast to that observed in microvilli of gland cells in the cabbage looper and tussock moth. A similarity of defensive gland cells to the cabbage looper gland cells is in the thickness of the basal lamina which underlies them. However, in the former insect, there is no differentiation into layers nor were haemocytes observed in close association with it.

Thus differences between the two types of gland cells could reflect differences in the biosynthetic pathways involved. These differences could occur by various methods: (a) the initial precursors may differ (b) the site of final steps in the biosynthesis may differ (c) the specific functions of the cells in the synthesis may differ. In consideration of alternative (b) - it appears from this study that the final forms of the pheromones are synthesized within gland cells or cuticle overlying them. Perhaps final steps in the synthesis of the defensive secretion occurs outside the cells and cuticle and within the lumen of the gland. This method occurs in the production of the defensive secretion in a carabid beetle. Enzymes necessary for the final steps in the synthesis of the defensive compound, are found within the lumen of the carabid reservoir (Schildknecht et al., 1970). The defensive secretion in this latter insect also consists of more than one

compound, although different in structure from those of the red-humped caterpillar. In consideration of alternative (c) - in cabbage looper gland cells the presence of lipid spheres is correlated with sex pheromone release but no such relationship has been deduced with field-collected red-humped caterpillars. Such interpretations require accurately-timed, laboratory-reared insects. However, it is possible that the defensive gland cells merely possess the capability of transferring pre-formed compounds from the haemolymph or haemocytes to the lumen of the gland. This may still involve alternative (b) in that a function of at least some of the gland cells could be the production and release of appropriate enzymes into the gland lumen.

Suggestions for Subsequent Investigations

The data derived from the present study was used to formulate several hypotheses concerning the structure of sex pheromone gland cells in relation to the production of pheromones. In many instances further experimentation using similar techniques was suggested from the results. In addition, the present study also revealed the necessity for further experimentation in at least three separate fields.

Electrophysiological studies could be initiated to determine the exact functions of muscles associated with glands. These studies should also attempt to decipher the inter-relationships between the nervous system, the hormonal system and the muscular system in controlling the eversion of glands and release of pheromone.

Organ culture of sex pheromone glands should be studied. Such glands properly maintained in an organ culture medium might prove to be an invaluable tool in the elucidation of biosynthetic pathways of

pheromones.

Immunochemical studies analogous to those used for the detection of glycolipids and phospholipids (Marcus and Schwarting, 1976), and followed by immunocytochemistry may prove useful. If these techniques can be applied to smaller lipids they may be used to ascertain whether there is a storage site of actual pheromones within gland cells or cuticle and, if so, to localize this site.

The analyses of the physical chemistry involved in the passage of lipids through artificially prepared membranes using, as lipids, the spruce budworm and cabbage looper pheromones, may provide answers related to the mode of exit of the pheromones through the gland cell membranes, and ultimately, through the cuticle.

Summary of Results

The sex pheromone glands of female spruce budworm, white-marked tussock moth and cabbage looper are modifications of the dorsal intersegmental membrane between the eighth and ninth abdominal segments.

Protrusion of the sex pheromone gland in the tussock moth and eversion of the glands in the other two species are controlled by at least five pairs of muscles which were identified and described. The surface of the gland cuticle in the spruce budworm was examined with the scanning electron microscope. It exhibits cuticular projections not present on cuticle from unmodified intersegmental membranes.

In pupae of the three species the developing gland cells are columnar. In the adults, the gland cells of the spruce budworm and the cabbage looper remain columnar whereas those of the tussock moth are goblet-shaped and are clustered around inward projections of endocuticle.

Gland cells of late pupae and young adults of all three species undergo changes in ultrastructure related to their development and ages of the insects. The modifications in the ultrastructure indicate that in adults these cells are primarily involved in lipid metabolism.

Mature gland cells in all three species contains smooth endoplasmic reticulum which is most extensively developed in gland cells of the tussock moth. Furthermore, in cells of this insect it is mainly cisternal whereas it is mainly tubular in the other two species. In all three species the apical membrane exhibits well-developed microvilli, each of which contains a tubule of smooth endoplasmic reticulum.

Lipid spheres appear in maturing gland cells of two species. In the spruce budworm they occur near the bases of cells and in the cabbage looper they occur throughout the cells. In cells of the latter insect there is a progressive development of lipid spheres which can be correlated with the age of the adult moth. In young adults these lipid spheres are all about the same size and evenly distributed throughout a cell. In older insects there is a definite disparity in size with larger spheres being located near the apices of a cell. In these latter cells lipid spheres have been observed which appear to be leaving the cell by everting the apical plasma membrane between microvilli.

Microperoxisomes were identified in gland cells of all three species. These are oval to elongate dilations of smooth tubular endoplasmic reticulum which exhibit a positive DAB reaction for catalase. In gland cells of the cabbage looper the lipid spheres are surrounded by microperoxisomes except when the spheres are in the eversions of the apical plasma membrane.

The lipid which leaves gland cells of the cabbage looper is stored as discrete deposits within the cuticle and was observed in both the light microscope and the electron microscope. The time of its appearance within the cuticle relative to the age of the insect suggests that the lipid contains the pheromone. In addition, tubular structures, distinct from epicuticular filaments, were observed, both within the cuticle and on its surface. No such modifications of the gland cuticle were observed in the spruce budworm and the tussock moth.

The basal lamina underlying sex pheromone gland cells of the cabbage looper is unusual and differs from that underlying gland cells of either of the other two species and from that underlying unmodified epidermal cells. Whereas, the basal lamina underlying the gland of the spruce budworm, the gland of the tussock moth and the unmodified epidermal cells of the cabbage looper, is thin and amorphous, that underlying the cabbage looper gland is thick and bi-layered. The layer facing the haemocoel (layer 2) contains ordered tubular structures which originate from granules contained in granular haemocytes. In addition, tubules from layer 2 cross the inner layer (layer 1) and are also reorganized near and within basal infolds of gland cells even at 36 hours before eclosion. Although haemocytes are observed near the basal lamina only in young adults, the structure of the basal lamina remains until the insects are at least 4 days old. The presence of many coated vesicles on the basal plasma membrane of gland cells in the vicinity of the re-organized portion of layer 2 in very young adults indicates that the cell is actively engaged in phagocytosing this material.

Lipid extracts of glands from cabbage loopers which had been injected with C^{14}-labelled compounds showed some incorporation of sodium acetate-$C_1{}^{14}$ and sodium acetate-$C_2{}^{14}$ but little, if any, incorporation of lauric acid-$C_1{}^{14}$. From the low incorporation of lauric acid-$C_1{}^{14}$ into gland lipids it is concluded that the last stage in the biosynthesis of the cabbage looper pheromone is not the desaturation and reduction of a 12C acid and the resultant acetylation of the corresponding alcohol. The low incorporation of sodium acetate into gland cell lipids, when the ultrastructural appearance of their cells indicates extensive lipid metabolism, suggests that the sodium acetate participates in the formation of the pheromone by combining with pre-existing compounds. These may be brought to gland cells from granular haemocytes via the basal lamina.

The defensive glands from the red-humped caterpillar was shown to contain n-decyl acetate as part of their defensive secretion. This is the first report of n-decyl acetate in the defensive secretion of lepidopteran larvae. The gland, situated ventrally in the thorax, opens medially at the posterior margin of the prothorax and consists of an anterior and posterior sac joined by an interglandular neck. The anterior sac contains 10X as much n-decyl acetate as the posterior sac. From ultrastructural observations of the gland cells it was determined that the cells do not contain cuticular ductules and that there are three or possibly four distinct types of cells within the gland. Two types, as suggested from their location, probably function to maintain the form of the orifice and the interglandular neck. The ultrastructure of the cells of the anterior and posterior sacs is similar and they have apical folds organized in a manner peculiar to this gland. The cells of

the anterior lateral longitudinal folds of the anterior sac contain large deposits of lipid. These cells were not observed without lipid deposits and lipid was not observed in the remaining cells of the anterior gland and those of the posterior gland, therefore, the suggestion is made that they are involved in the production of the n-decyl acetate and/or its release into the lumen of the gland.

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TABLES

Table I: Muscles associated with the sex attractant glands and their probable functions in eversion (or protrusion) and inversion of the glands.

Group	Type of abdominal muscle [1]	Function ascribed by Snodgrass (1935)	Function as related to gland	Spruce budworm	Cabbage looper	Tussock moth
1	internal dorsals	retractor of abdomen	inversion of gland	1 pr	2 pr	4 pr
1 p	internal dorsals	retractor of abdomen	inversion of gland	1 pr	1 pr	1 pr
2	reversed external dorsals	protractor of abdomen	eversion (or protrusion) of gland	1 pr	1 pr	absent
2 p	reversed external dorsals	protractor of abdomen	eversion (or protrusion) of gland	1 pr	1 pr	1 pr
3	laterals	compressor of abdomen	eversion of gland	1 pr	2 pr	absent
3 p	laterals	compressor of abdomen	eversion (or protrusion) of gland	1 pr	1 pr	1 pr
4	internal dorsals	retractor of abdomen	inversion of gland	1 pr	1 pr	1 pr
5	laterals	compressor of abdomen	eversion (or protrusion) of gland	1 pr	-	1 pr

[1] Accepted terminology for these muscles of abdominal segments (Snodgrass, 1935).

Table II: Definition of headings

Compound injected:	The radioactively labelled compound injected into an insect.
Portion of insect:	For purposes of extraction and counting each insect was divided into three portions: head, thorax and wings (htw), abdomen (abd) and gland (gl).
Incubation time:	Time from injection until the insect was killed. The times were either 8 hours or 3 days.
Lipid extract recovery:	Disintegrations per minute (dpm) of labelled lipids extracted from each insect portion. The lipids were extracted with chloroform/methanol (2:1).
Residue recovery:	Dpm of labelled compounds remaining in tissues of a portion of insect after each portion was extracted with chloroform/methanol.
Total portion recovery:	The total dpm recovered from each portion of insect (Lipid extract recovery + residue recovery).
Total C^{14} recovered	The total dpm recovered from each insect i.e., htw + abd + gl.
Percent recovery (Column 4):	$\frac{\text{Total portion recovery}}{\text{Total } C^{14} \text{ recovered per insect}} \times 100\%$

Table II Incorporation of C^{14} labelled compounds into body tissues of the cabbage looper moth after 8 hrs or 3 days post-treatment and expressed in disintegrations per minute (dpm).

Compound injected	portion of insect	incubation time	1 Lipid extract recovery	2 Residue recovery	3 Total portion recovery (Sum of 1 + 2	4 C^{14} recovered as % of total recovered
	htw.	8h	20198	2713	22911	13.8
		3d	44188	6645	50833	62.8
lauric acid-C_1^{14}	abd.	8h	133628	9356	142984	85.9
		3d	27233	2562	29795	36.8
amount injected						
350,900 dpm/insect	gl.	8h	414	98	512	0.3
		3d	262	88	350	0.4
Total C^{14} recovered		8h			166407	
		3d			80978	
	htw.	8h	13642	4882	18524	48.5
		3d	20553	9816	30369	44.9
sodium acetate-C_1^{14}	abd.	8h	12102	6487	18589	48.7
		3d	28234	8414	36648	54.J
amount injected						
788,900 dpm/insect	gl.	8h	820	244	1064	2.8
		3d	509	181	690	1.0
Total C^{14} recovered		8h			3[illegible]77	
		3d			67707	
	htw.	8h	40942	23184	64126	52.8
		3d	16698	9108	25806	42.5
sodium acetate-C_2^{14}	abd.	8h	38992	14924	53916	44.4
		3d	26453	6810	33263	54.8
amount injected						
644,900 dpm/insect	gl.	8h	2421	954	3375	2.8
		3d	750	855	1605	2.6
Total C^{14} recovered		8h			121417	
		3d			60674	

Table III. Body weights of the three portions of cabbage looper adults used in labelling experiments. These portions are head, thorax and wings (htw) abdomen (abd) and gland (gl).

	htw*	abd.*	gl.*
	94.98	99.8	0.65
	122.35	102.4	0.55
	96.10	63.85	0.40
	157.60	86.30	0.50
	110.90	91.05	0.35
Average weight of an insect portion	58.2	44.3	0.24
% of total body weight	56.5%	43.1%	0.23%

*These figures represent 5 replicates of weights (mg.) of the body portions from two insects.

Table IV. Percentages of labelled compounds incorporated into lipid and other components of portions of cabbage loopers.

Compound injected	portion of insect	incubation time	1 Total C^{14} incorporated as percent of C^{14} injected	2 C^{14} incorporated into lipid as % of C^{14} injected	3 C^{14} in lipid as % of total C^{14} recovered
lauric acid-C_1^{14}	htw	8h	6.5	5.8	13.1
		3d	14.5	12.6	61.6
	abd	8h	40.7	38.1	86.7
		3d	8.5	7.8	38.0
	gl.	8h	0.1	0.1	0.2
		3d	0.1	0.1	0.4
Total C^{14} incorporated per insect (%)		8h	47.3	44.0	
		3d	23.1	21.5	
sodium acetate-C_1^{14}	htw	8h	2.3	1.7	51.4
		3d	3.8	2.6	41.7
	abd	8h	2.4	1.5	45.4
		3d	4.6	3.6	57.3
	gl.	8h	0.1	0.1	3.1
		3d	0.1	0.1	1.0
Total C^{14} incorporated per insect (%)		8h	4.8	3.3	
		3d	8.5	6.3	
sodium acetate-C_2^{14}	htw	8h	9.9	6.3	49.7
		3d	4.0	2.6	38.0
	abd	8h	8.4	6.0	47.3
		3d	5.2	4.1	60.2
	gl.	8h	0.5	0.4	3.0
		3d	0.2	0.1	1.7
Total C^{14} incorporated per insect (%)			18.8	12.7	
			9.4	6.8	

FIGURES

Fig. 1. Position adopted by calling insects
(a) spruce budworm (b) cabbage looper (c) white marked tussock moth. Arrows point to sex pheromone glands.

Fig. 1

Fig. 2(a). Whole mount of sex pheromone gland from resting tussock moth. The gland (gl) is not protruded and is represented by a crescent-shaped dorsal fold underneath the eighth tergite (8). Stained with Feulgen's reagent. 40X.
Gland, gl; ovipositors, o; posterior apophysis, pa; muscle 2 p, 2 p; muscle 4, 4; seventh tergite, 7; eighth tergite, 8.

(b). Whole mount of protruded sex pheromone gland from injected insect. When the gland (gl) is protruded it is represented as a rectangular dorsal field extending posterior to the eighth tergite (8). Stained with Feulgen's reagent. 40X.
Anterior apophysis, aa; gland, gl; ovipositors, o; posterior apophysis, pa; seventh tergite, 7; eighth tergite, 8.

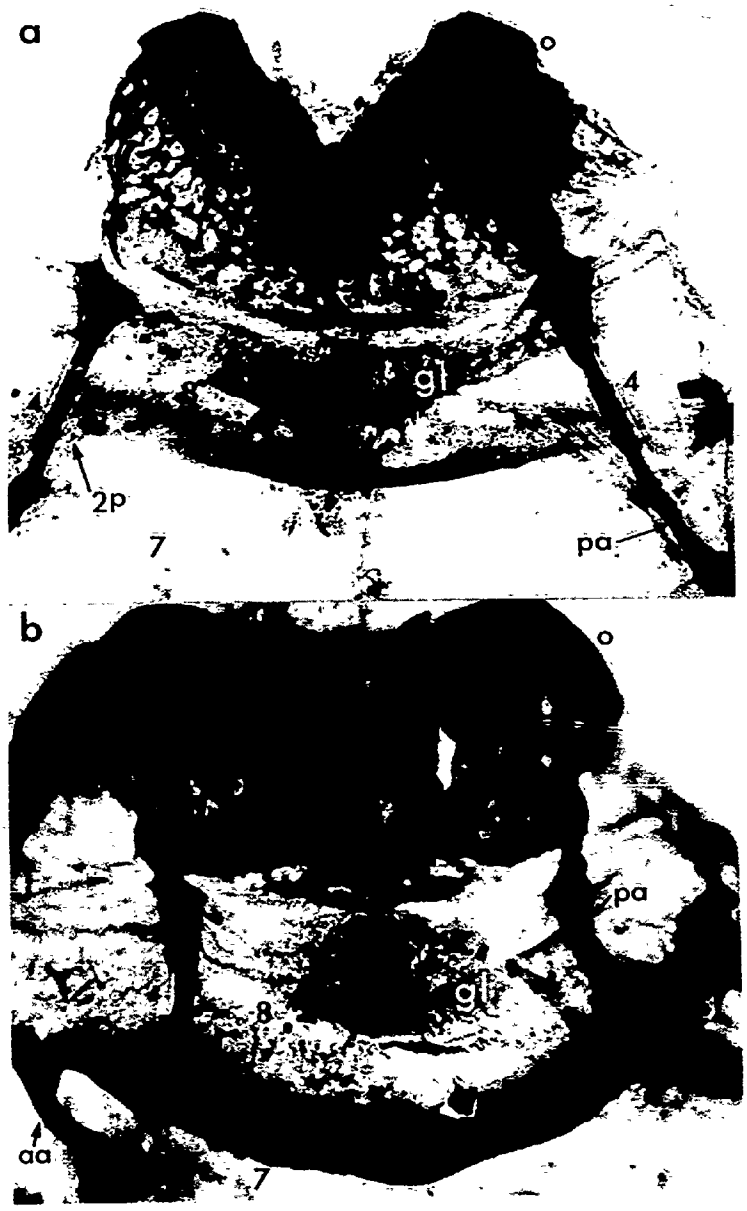


Fig. 2

Fig. 3. Topography of the spruce budworm sex pheromone gland. Scanning electron microscopy. (a) Partially everted gland (gl) which is the dorsal intersegmental membrane between the eighth (8) and ninth abdominal segments. (The ninth segment is represented here by the ovipositors (o)). The area enclosed in the rectangle is enlarged in Fig. 3c and that enclosed in the circle is enlarged in Fig. 3d. 75X.
(b) The unmodified or non-glandular intersegmental membrane (i) between the seventh (7) and eighth (8) abdominal segments. The area enclosed in the rectangle is enlarged in Fig. 3e. 75X
(c) Anterior portion of gland cuticle showing cuticular projections of 'spikes' (arrows) on surface. 2250X
(d) Posterior portion of gland surface illustrating lack of 'spikes' on its surface. 2250X
(e) Surface of unmodified intersegmental membrane. Note lack of 'spikes'. 2400X

Fig.3

Fig. 4. Whole mounts of terminal abdominal segments illustrating apophyses and muscles associated with the glands. These whole mounts represent one side of each insect thus illustrating only one member of each pair of muscles. The black numbers on white background designate the groups of muscles.
(a) Spruce budworm with gland inverted. The groups of muscles illustrated are 1, 1 p, 3, 5. 50X.
(b) Spruce budworm with gland everted. The groups of muscles illustrated are 1, 1 p, 2, 2 p, 4. The anterior termination of the anterior apophyses (aa) is designated by double arrows. 50X.
(c) Cabbage looper with gland everted. The groups of muscles illustrated are 1 (a and b), 1 p, 2, 2p, 3, 4. The anterior termination of the anterior apophyses. (aa) is designated by double arrows. 50X.
(d) Tussock moth with gland inverted. The groups of muscles illustrated are 1 (a, b, c and d), 1 p, 2 p, 4, 5. (Muscle 3 p lies adjacent to 4 and cannot be distinguished in this figure - (see Fig. 6). 20X.

Anterior apophysis, aa; gland, gl; ovipositors, o; posterior apophysis, pa. The numbers 7 and 8 designate the seventh and eighth abdominal segments.

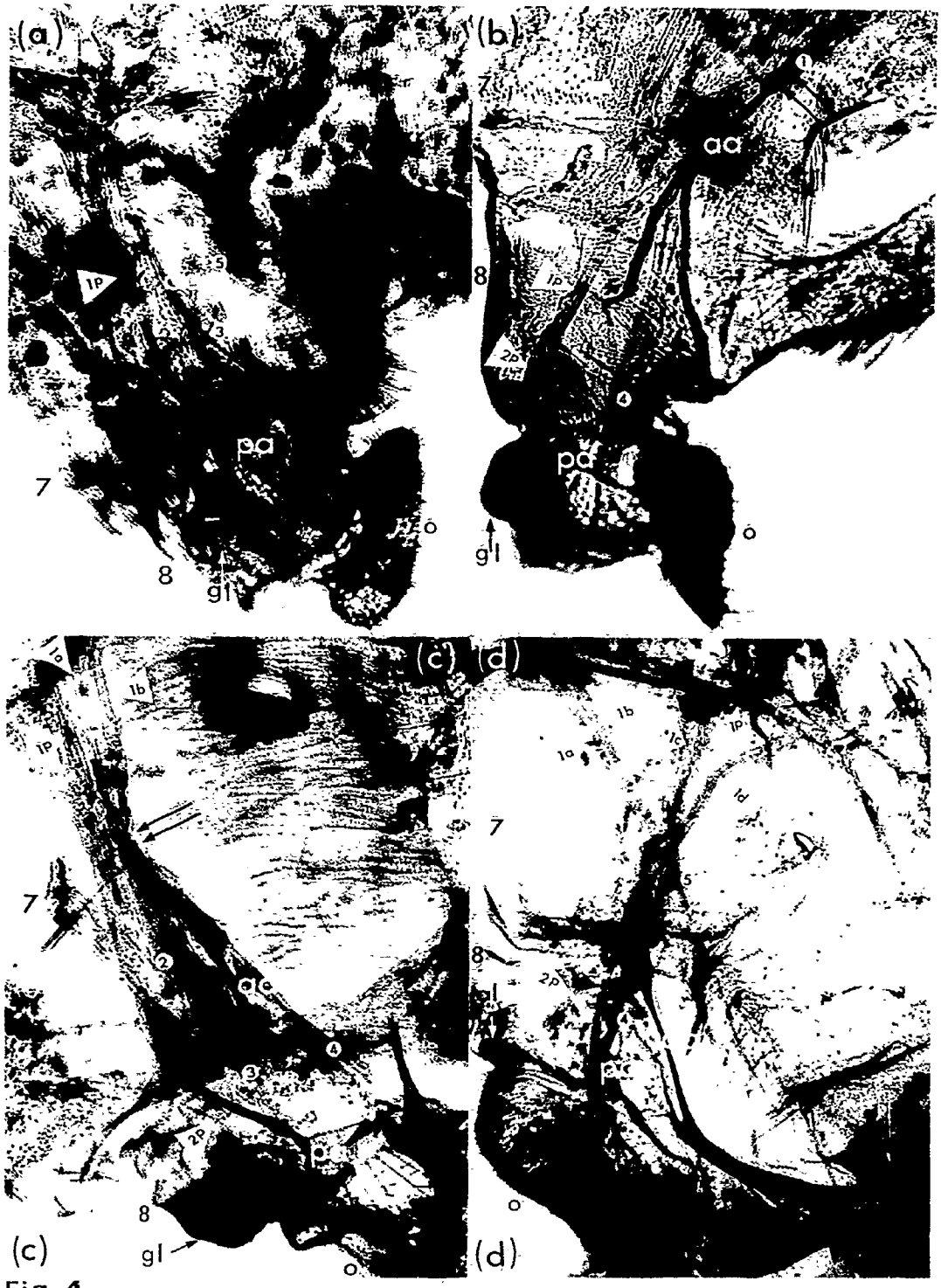


Fig. 4

Fig. 5. Whole mounts of terminal abdominal segments showing points of attachment (→ ←) of posterior apophyses (pa) to the glands* (gl). The encircled area in each figure represents the origin of the posterior apophyses on the ovipositors (o).

(a) Spruce budworm. Muscles of group 4 are illustrated (black number on white background) stained with borax carmine. 90X.

(b) Cabbage looper. Muscles of group 4 are illustrated. Reacted with diaminobenzidine and with gland cells removed. 60X.

(c) Tussock moth. Muscles of group 4 are illustrated. Stained with borax carmine. 115X.

The numbers 7 and 8 represent, respectively, the seventh and eighth abdominal segments.

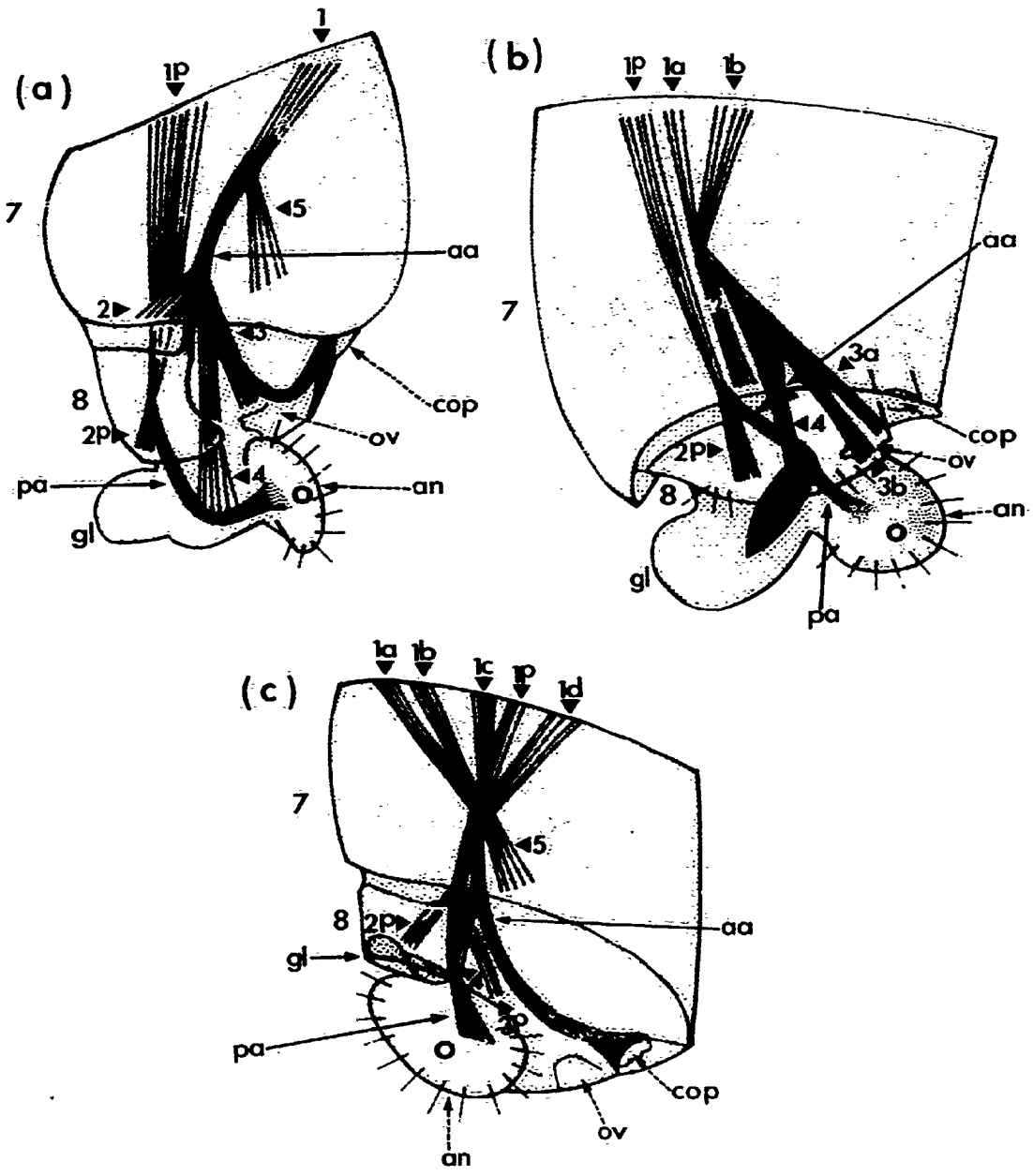
*Only the posterior apophyses for the right half of each insect is shown although there are identical structures on left half.

Fig·5

Fig. 6. Semidiagrammatic interpretation of muscles and apophyses (aa and pa) associated with sex pheromone glands (gl) as viewed laterally and illustrating only the right member of each pair.
(a) Spruce budworm. Groups of muscles illustrated are 1, 1 p, 2, 2 p, 3, 4, 5.
(b) Cabbage looper. Groups of muscles illustrated are 1 (a and b), 1 p, 2, 2 p, 3 (a and b), 4.
(c) Tussock moth. Groups of muscles illustrated are 1 (a, b, c, and d), 1 p, 2 p, 3 p, 4, 5.

Anterior apophysis, aa; gland, gl; ovipositor, o; posterior apophysis, pa. The numbers 7 and 8 represent the seventh and eighth abdominal segments.

The broken arrows indicate positions of the anus (an); copulatory opening (co) and ovipore (ov). These are out of the plane of these drawings.


(a)
1
1P
7
5
aa
2
3
8
cop
2P
ov
pa
4
an
gl
(b)
1P
1a
1b
aa
7
3a
4
cop
2P
ov
8
3b
an
gl
pa
(c)
1a
1b
1c
1P
1d
7
5
8
2P
aa
gl
4
pa
cop
ov
an

Fig· 6

Fig. 7. Spruce budworm. Apical region of developing gland cell showing disorganised microvilli (mv) and short segments of rough endoplasmic reticulum (rer) 35,350X. Age, 1 to 2 days pre-eclosion. Coated vesicle, cv; Golgi complex, G; multivesicular body, mvb.

Fig. 8. Spruce budworm. Basal region of developing gland cell showing profiles of rough endoplasmic reticulum (rer) longer than those observed in the apical region. In the basal region the rer is oriented along the longitudinal axis of a cell. 25,000X. Age, 1 to 2 days pre-eclosion. Basal involution, bi; Golgi complex, G; intercellular space, is.

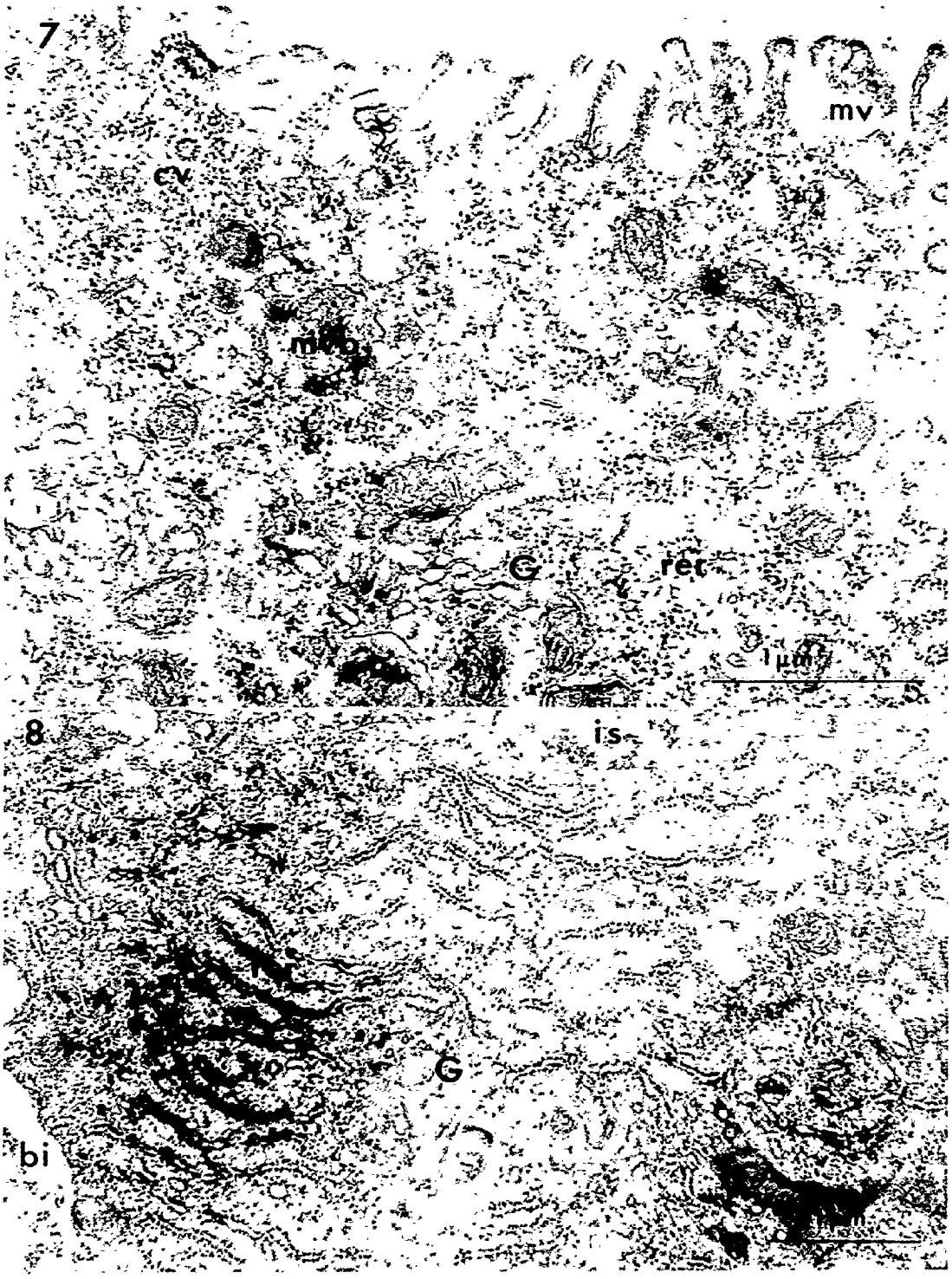

7
mv
cv
mvb
G
ret
1 µm
is
8
G
bi

Fig. 9. Tussock moth. Apical region of developing gland cell illustrating the folds (af) of the apical plasma membrane and the overlying cuticle. 23,600X. Age, 1 day pre-emergence. Coated vesicle, cv; dense epicuticle, de; dense plaque, dp; lamellate endocuticle, en; pore canal containing epicuticular filaments, pc.

Insert: Thick section embedded in Araldite, stained with toluidine blue. 400X. Cuticle, c; nucleus, n.

Fig. 10. Tussock moth. Mid region of developing gland cell illustrating the organization of the rough endoplasmic reticulum (rer). 16,000X. Age, 1 day pre-emergence. Golgi complex, G; microtubules, mt; nucleus, n.


9
c
de
n
30µm
pc
GV
1µm
10
n
rer
G
n
mi
1µm

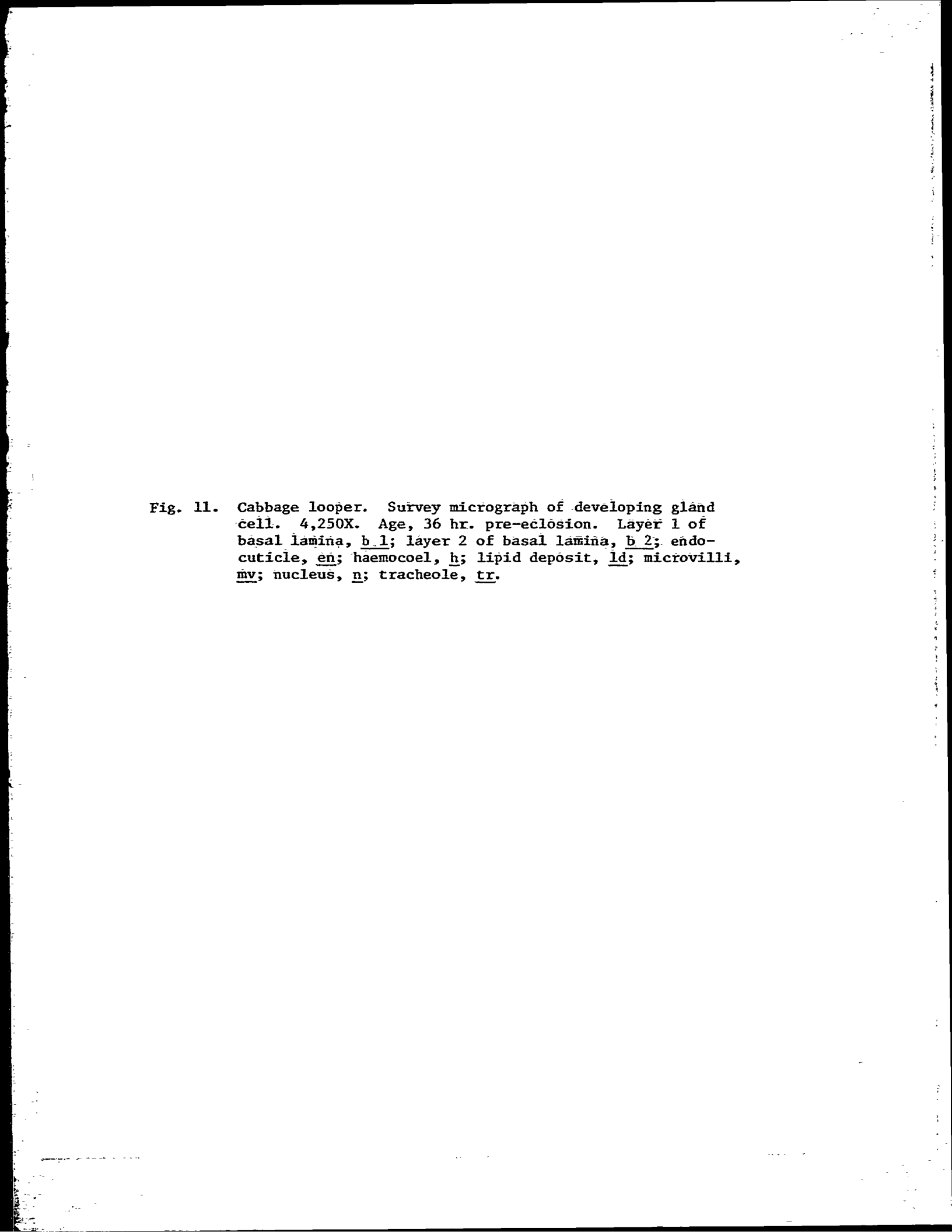


Fig. 11. Cabbage looper. Survey micrograph of developing gland cell. 4,250X. Age, 36 hr. pre-eclosion. Layer 1 of basal lamina, b 1; layer 2 of basal lamina, b 2; endocuticle, en; haemocoel, h; lipid deposit, ld; microvilli, mv; nucleus, n; tracheole, tr.

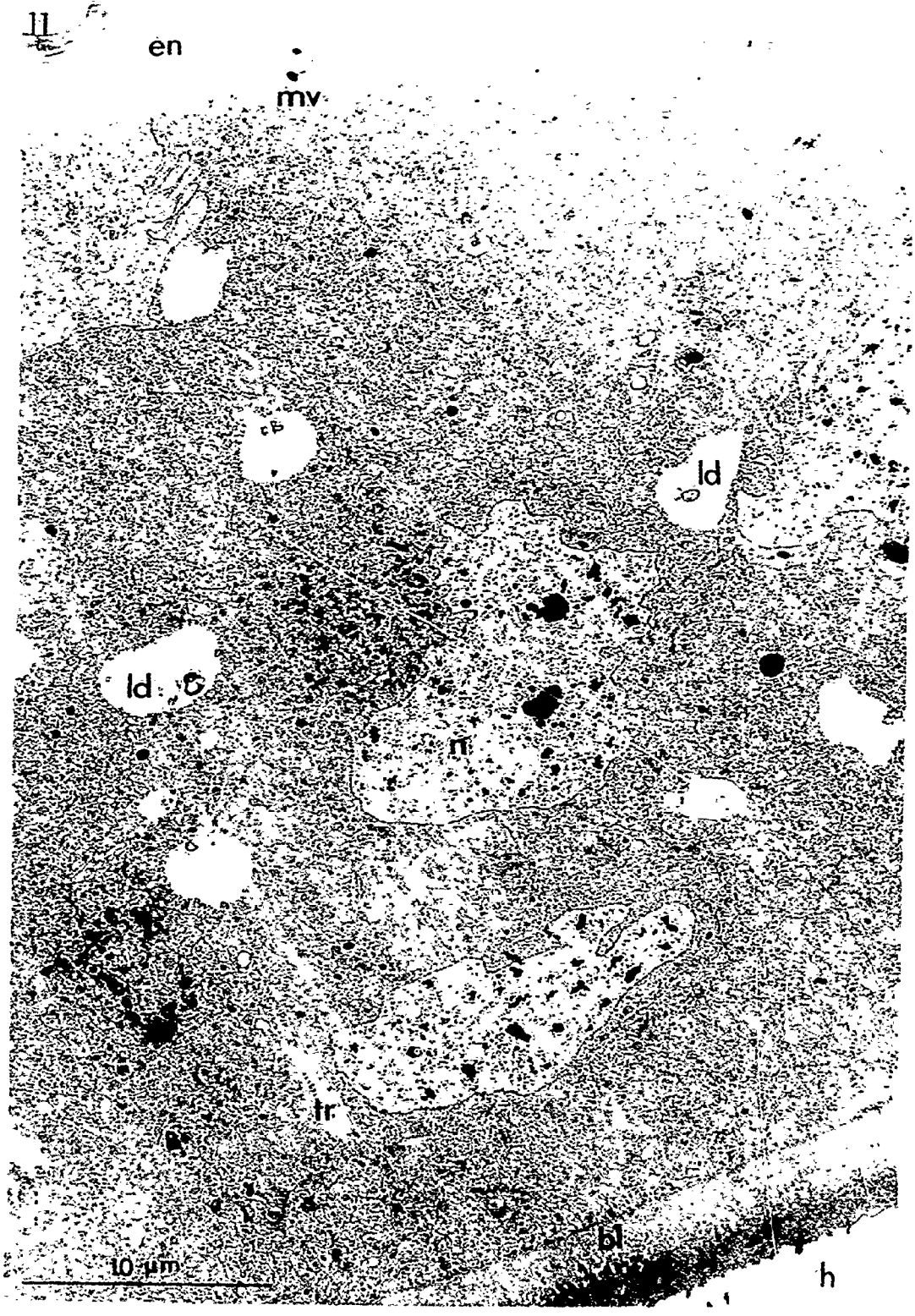

11
en
mv
ld
ld
n
tr
bl
h
10 µm

Fig. 12. Spruce budworm. Epicuticular filaments in the cuticle of developing gland cells. The pore canals are not visible because they do not contain many epicuticular filaments.* 38,850X. Age, 1 to 2 days pre-emergence. Dense epicuticle, de; endocuticle, en; epicuticular filaments, ef; inner cuticulin, ic; microvilli, mv; outer cuticulin, oc. Open arrow designates a pore in inner cuticulin.

Fig. 13. Spruce budworm. Pore canals in cuticle of adult gland cells. The parabolic pattern is produced by the pore canals following the helicoidal arrangement of microfibrils in the endocuticle. At this magnification the pore canals are visible only because they contain the epicuticular filaments. 38,850X. Age, EC+ 46 $\pm$ 1 1/2 hr. Cuticulin, cu; dense epicuticle, de; epicuticular filaments, ef; filamentous structure, f; pore canal, pc.

*For a higher magnification of the epicuticular filaments (ef), the reader is referred to Fig. 34.


12
oc
ic
cu
de
de
ef
pc
en
f
m
1µm
1µm

Fig. 14. A composite diagram illustrating the features common to developing gland and epidermal cells of all three species. Microvilli and apical folds are both present on the apical plasma membrane. Basement membrane, bm; coated vesicle, cv; dense epicuticle, de; endocuticle, en; epicuticular filaments, ef; Golgi complex, G; inner cuticulin, ic; intercellular space, is; junctional area, j; mitochondrion, m; microtubule, mt; multivesicular body, mvb; nucleus, n; outer cuticulin, oc; pore, p; rough endoplasmic reticulum rer.

14

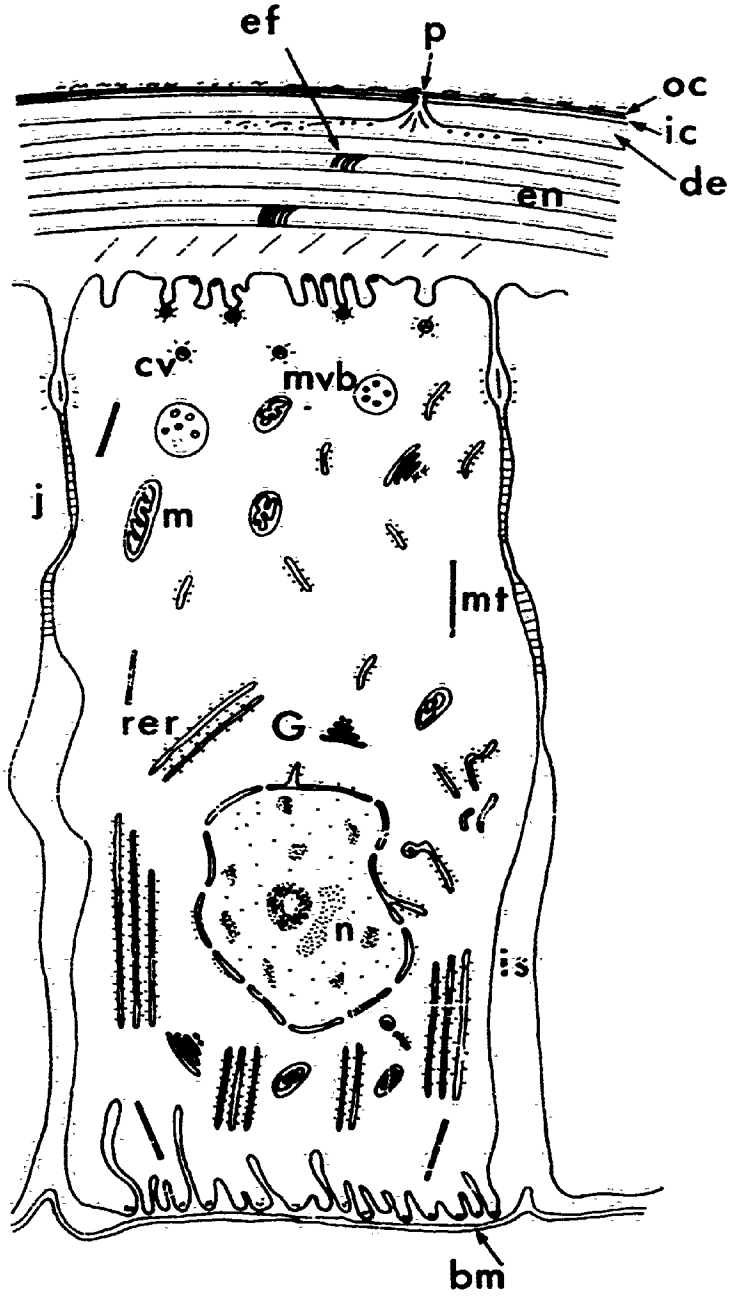
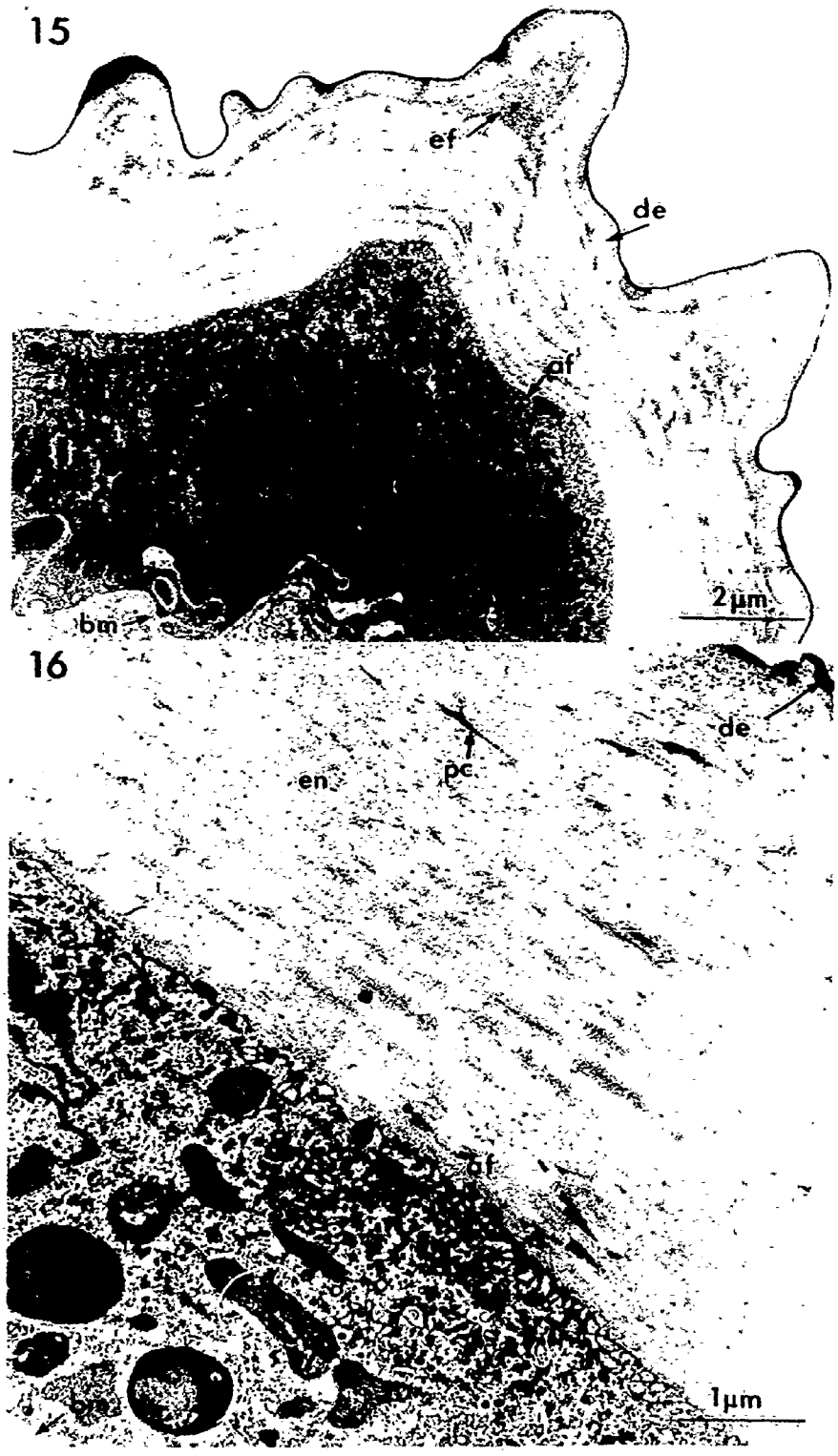


Fig. 15. Spruce budworm. Epidermal cell from unmodified intersegmental membrane. 9,650X. Age, EC+ 15±1 hr. Apical fold, af; basal lamina, bm; dense epicuticle, de; epicuticular filaments, ef; nucleus, n.

Fig. 16. Tussock moth. Epidermal cell from unmodified intersegmental membrane. 20,500X. Age, EC+ 24 hr. Apical fold, af; basal lamina, bm; dense epicuticle, de; lamellate endocuticle, en; mitochondrion, m; nucleus, n; pore canals, p.


15
ef
de
af
2µm
bm
16
de
en
pc
af
1µm

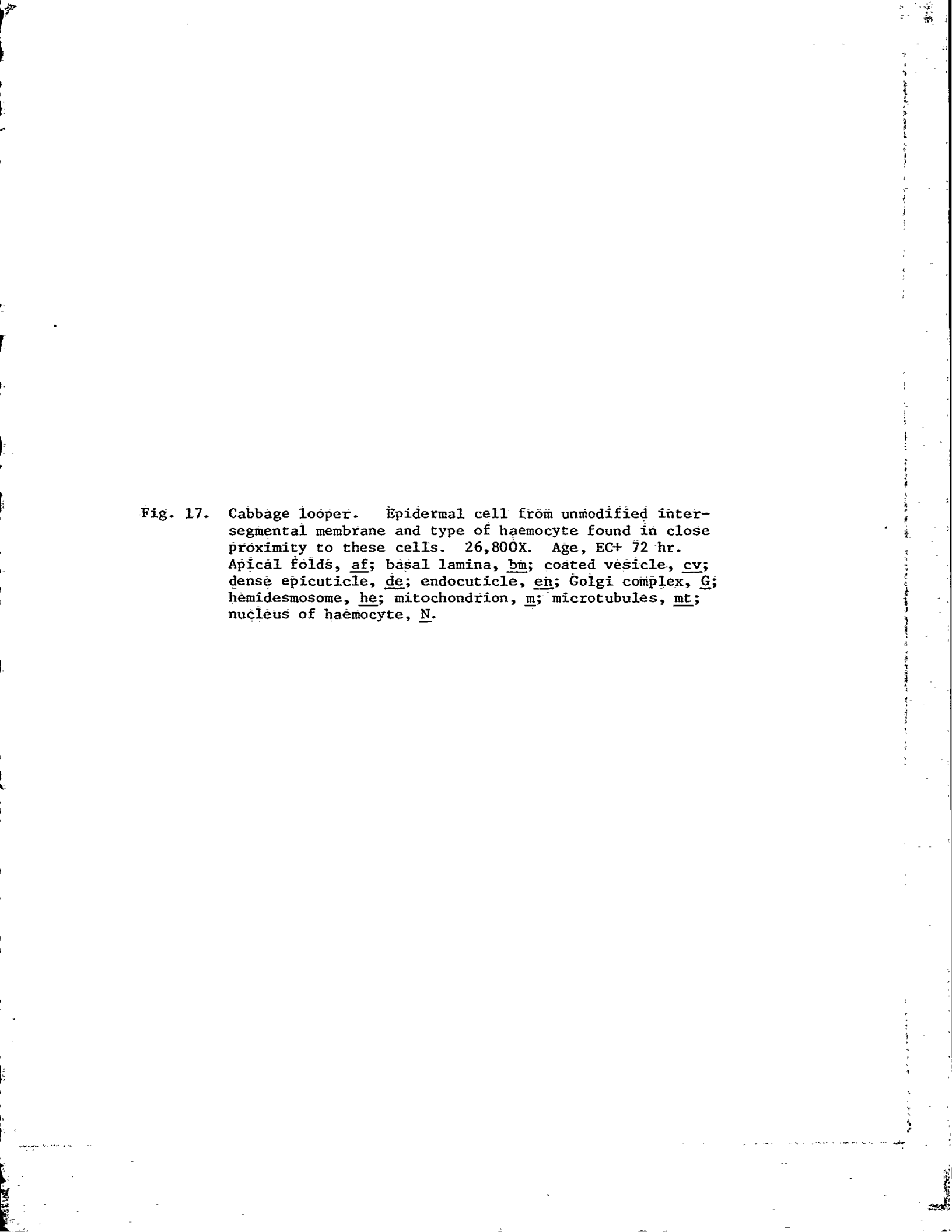


Fig. 17. Cabbage looper. Epidermal cell from unmodified intersegmental membrane and type of haemocyte found in close proximity to these cells. 26,800X. Age, EC+ 72 hr. Apical folds, af; basal lamina, bm; coated vesicle, cv; dense epicuticle, de; endocuticle, en; Golgi complex, G; hemidesmosome, he; mitochondrion, m; microtubules, mt; nucleus of haemocyte, N.

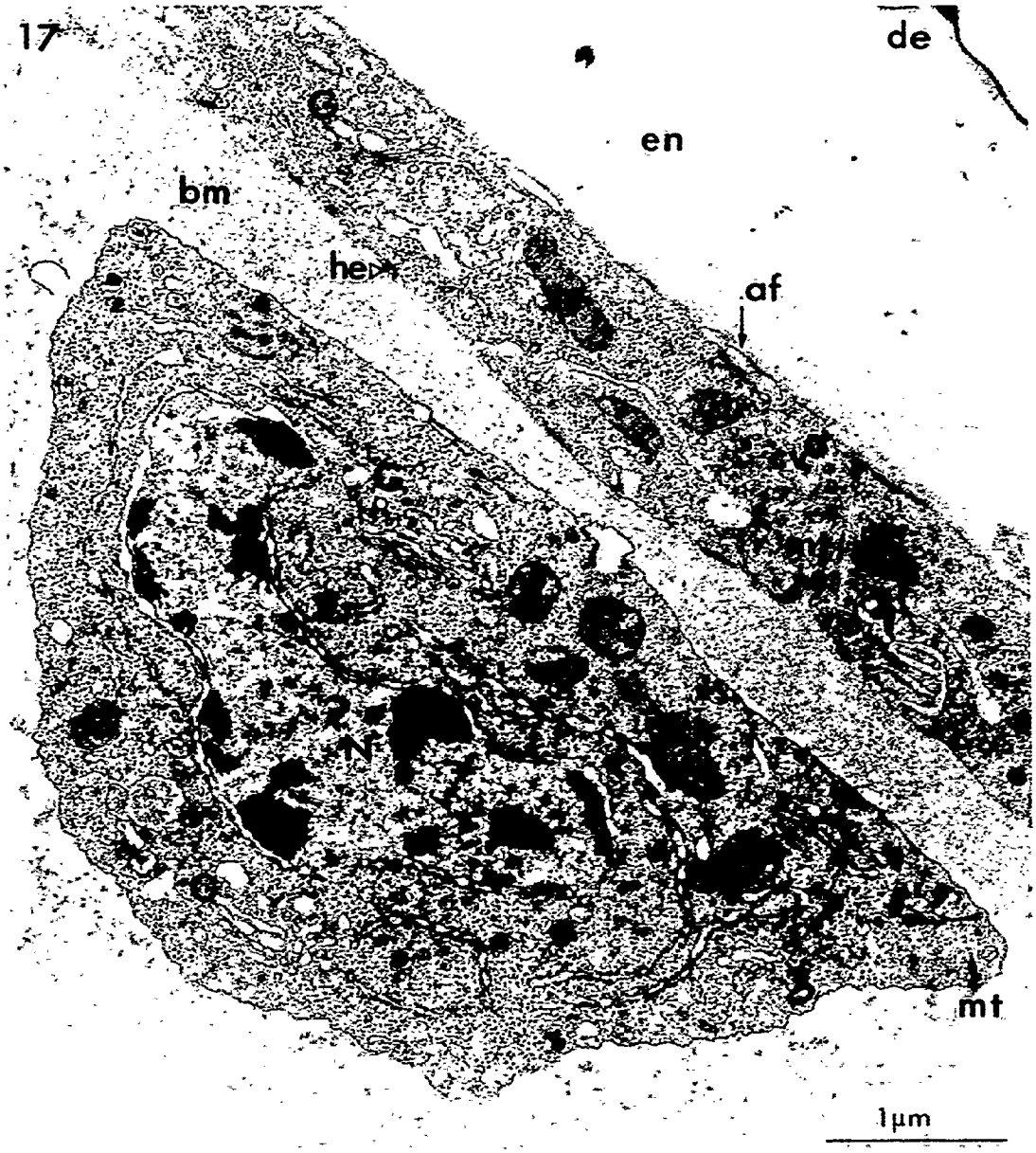

17
de
en
bm
af
N
mt
1µm

Fig. 18. The interpretation of generalized adult epidermal cell from an unmodified intersegmental membrane. Apical folds, af; basal lamina, bm; dense epicuticle, de; endocuticle, en; epicuticular filaments, ef; Golgi complex, G; hemidesmosome, he; junctional area, j; microtubule, mt; mitochondrion, m; multivesicular body, mvb; nucleus, n.

18

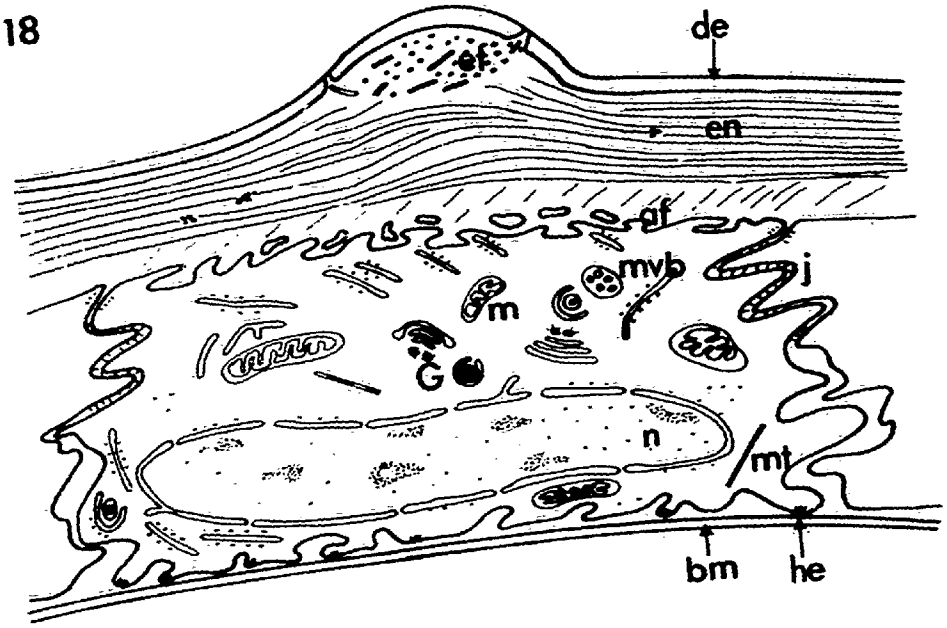


Fig. 19. Spruce budworm. Adult gland cells. There is no variation in size, number or distribution of lipid spheres within cells of insects from 1 day to 3 days of age. 4600X. Age, EC+ 46+1 1/2 hr. Basal infolds, bi; basal lamina, bm; dense epicuticle, de; epicuticular filaments, ef; endocuticle, en; glycogen deposit, gd; lipid sphere, li; microvilli, mv.

Insert represents glycogen deposit such as represented in lower right-hand corner and observed at higher magnification. 9700X. Age, EC+ 46+1 1/2 hr.


19
de
ef
mv
en
gd
li
gd
bi
bm
5 µm

Fig. 20. Tussock moth. Maturing gland cells and overlying cuticle. Note the appearance of the cuticular extensions (ce). 15,375X. Age, 10 hr. pre-eclosion. Cuticular extensions, ce; dense epicuticle, de; lamellate endocuticle, en; epicuticular filaments, ef; microvilli, mv; pore canal, pc.

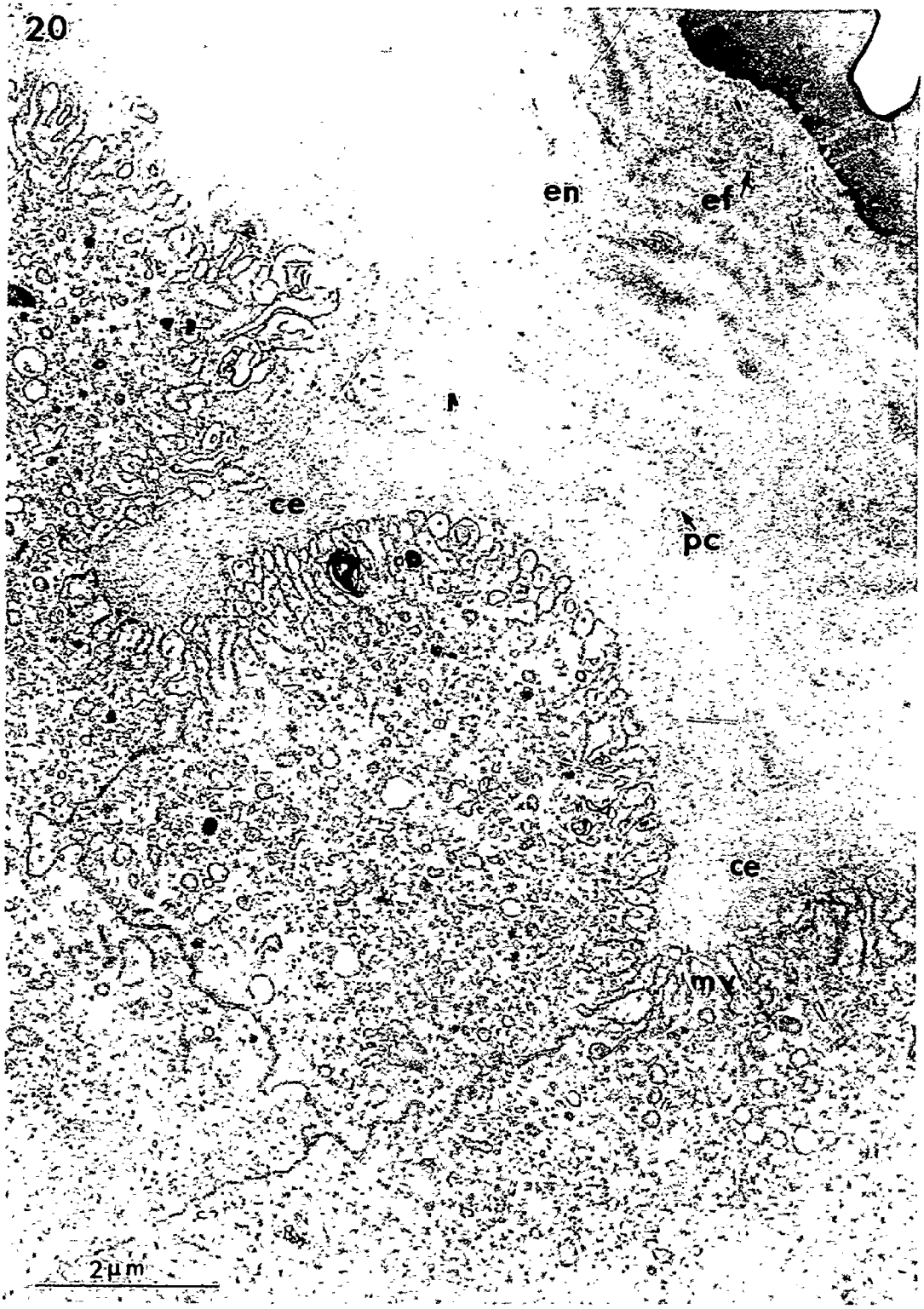

20
en
ef
ce
pc
ce
mx
2µm

Fig. 21. Tussock moth. Portion of one lobe of adult pheromone gland showing goblet-shaped cells and overlying cuticle. Note the absence of lipid spheres. 3,100X. Age, EC+72±3 hr. Basal lamina, bm; cuticular extension, ce; lamellate endocuticle, en; microvilli, mv; nucleus, n; pore canal, pc; protein granules, pg.

Insert: Thick cross section through gland showing several lobes. 180X. Age, 2 days. Cuticle, c; nucleus, n; underlying tracheae, tr.

For a higher magnification of pore canals and their contents, the reader is referred to Fig. 41.

21

Fig. 22. Cabbage looper. Maturing gland cell illustrating appearance of lipid in newly-eclosed adult cells. 4875X. Age, EC+1 hr. Endocuticle, en; lipid sphere, li; mitochondria, m; microbodies, mb; microvilli, mv; nucleus, n; tracheole, tr. Layer 1 of basal lamina, b1; layer 2 of basal lamina, b2.


22
en
mv
lr
tr
tr
b1
b2
3µm

Fig. 23. Cabbage looper. Mature (adult) gland cell illustrating the distribution of lipid spheres. 5625X. Age, EC+24 hr. Endocuticle, en; lipid spheres, li; mitochondria, m; microbodies, mb; microvilli, mv; nucleus, n; tracheole, tr. Layer 1 of basal lamina, bl; layer 2 of basal lamina b2.

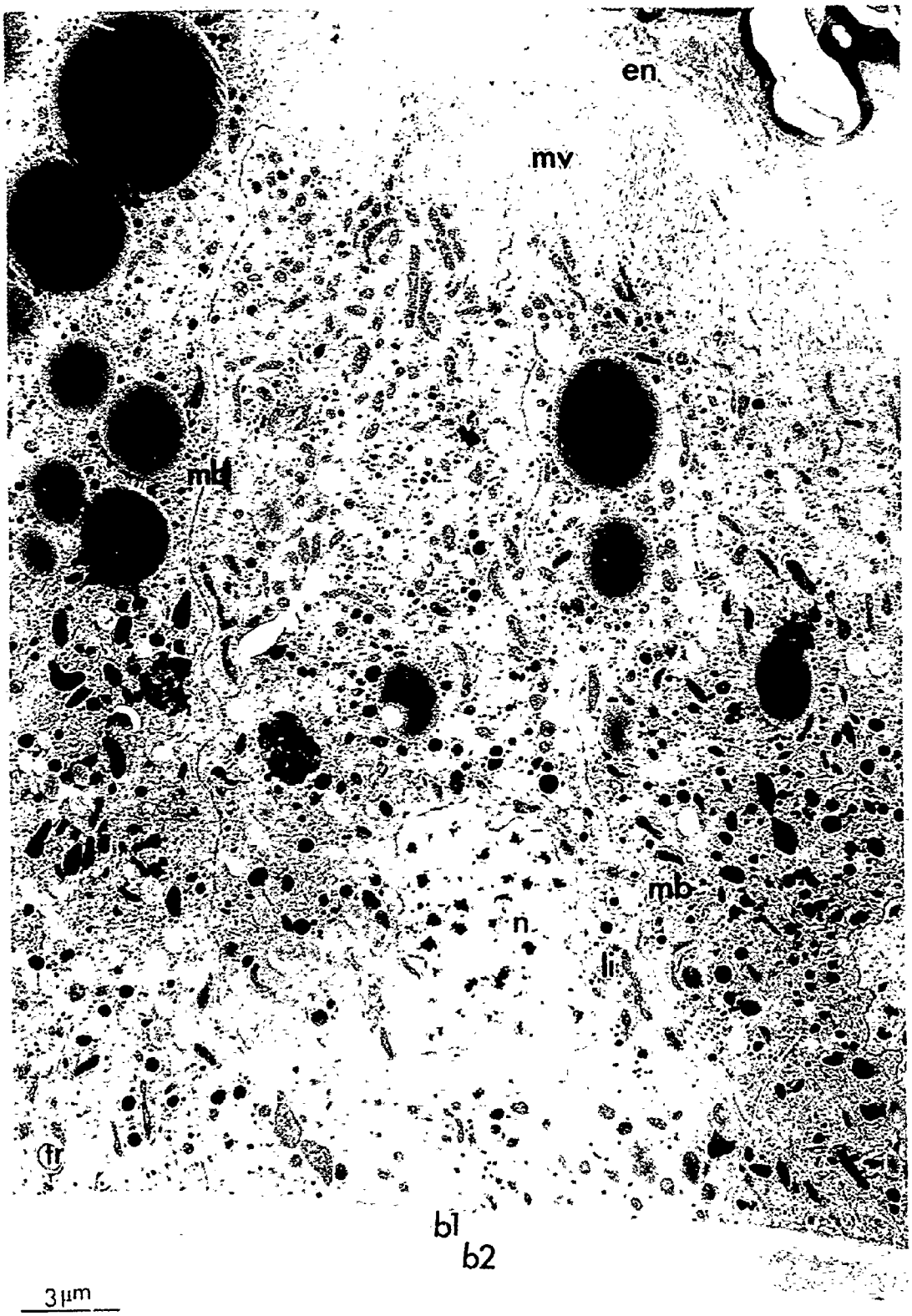

en
mv
mb
mb
n
li
fr
b1
b2
3 µm

Fig. 24. Cabbage looper. The size and distribution of lipid in developing and adult gland cells. Dense areas within cells and cuticle represent lipid deposits as drawn from light-microscope sections. The size and numbers per section were determined from longitudinal sections of 10 different cells. Basal lamina, bm; cuticle, c; microvillate surface, mv.

(a) Age: 36 hr. - 1 hr. Pre-EC
Size of lipid: variable 5 µm
No. of lipid spheres per section: 3-5
Insect pheromone content: undetermined (from Shorey et al. 1978)[1]

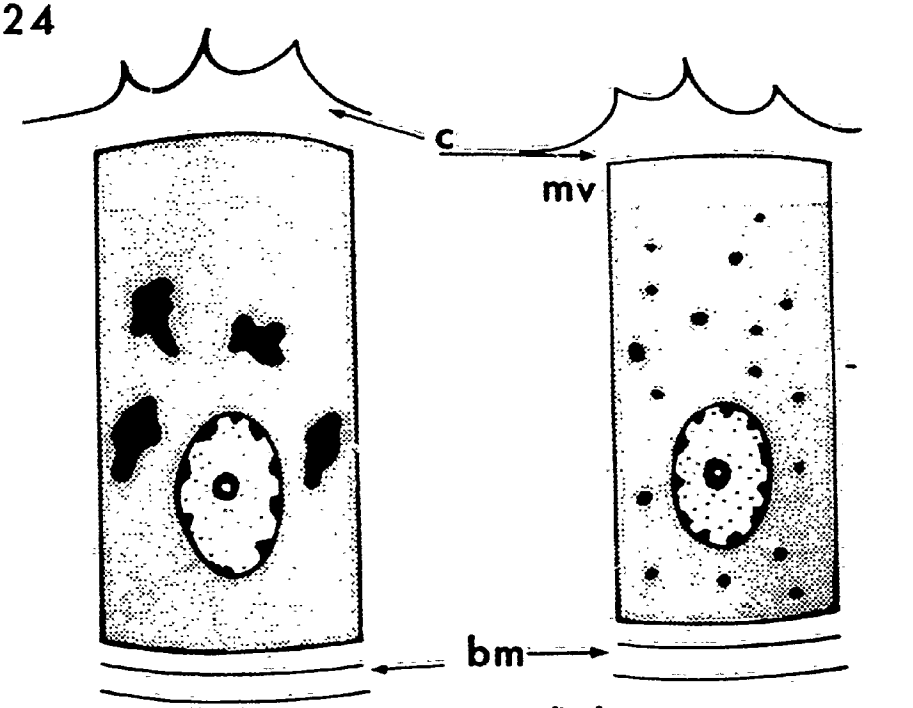
(b) Age: EC + (1-3) hr.
Size of lipid: 0.5-2.5 µm
No. of lipid spheres per section: 10-25
Insect pheromone content: 10%[2] (from Shorey et al. 1968)

(c) Age: EC + (6-12) hr.
Size of lipid spheres: 0.5 - 5 µm
No. of lipid spheres per section:
Insect pheromone content: 10% - 10% (from Shorey et al. 1968)

(d) Age: EC + (18-141) hr.
Size of lipid: Base to usually 0.5 - 1 µm
Nucleus to apex 1 - 10 µm
No. of lipid spheres per section: 50 - 100
Insect pheromone content: 10% (EM + 24 hr) to 100% (EM + 96 hr) (from Shorety et al. 1968)

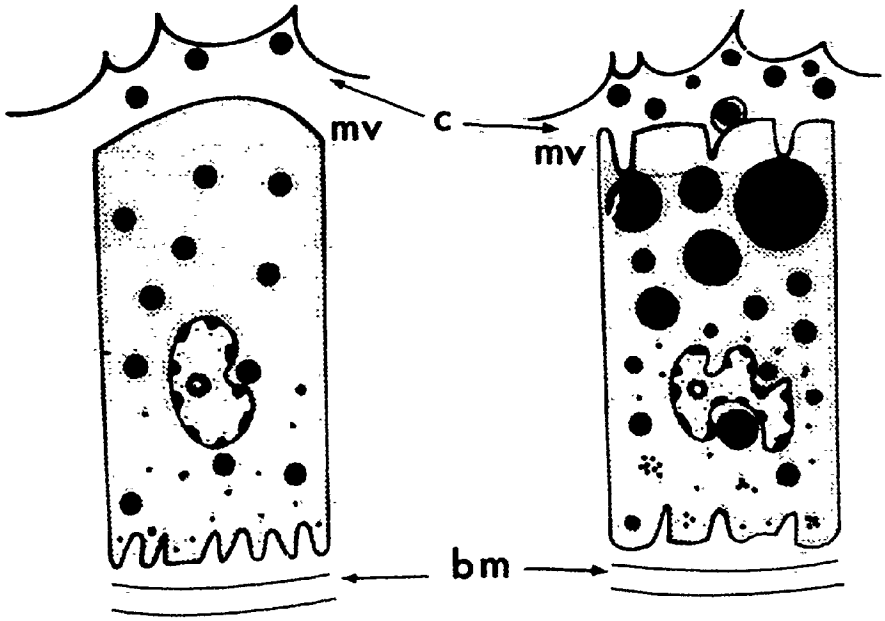
1) there is no pheromone in pupae; as determined by behavioural bioassays in a previous study (Shorety and Gaston, 1965).

2) the maximum amount of pheromone is contained in 2 to 4-day-old adults and is designated as 100%.



(a) 36 hr - 1 hr pre-EC

(b) EC + (1-3) hr



(c) EC + (6-12) hr

(d) EC + (18-141) hr

Fig. 25. Appearance of cuticle which overlies maturing gland cell. Note the appearance of tubules ('cores'-co) within the microvilli. 23,700X. Age, EC + 5 hr. Dense epicuticle, de; epicuticular filaments, ef; endocuticle, en; microvilli, mv. Double arrows indicate longitudinal sections through spikes.


25
de
ef
en
co
mv
1 µm

Fig. 26. Cabbage looper. Deposits of lipid in cuticle overlying mature or adult gland cell. 15,750X. Age, EC+91 hr. Dense epicuticle, de; endocuticle, en; cuticular lipid, cl; microvilli, mv.

26

Fig. 27. Spruce budworm. Basal region of adult gland cell illustrating close association of mitochondria with lipid spheres. 30,800X. Age, EC+ 46±1 1/2 hr. Golgi complex, G; lipid sphere, li; mitochondria, m; rough endoplasmic reticulum, rer; smooth endoplasmic reticulum, ser.

Fig. 28. Spruce budworm. Basal region of adult gland cell illustrating the large basal involutions (bi), basal coated vesicle, (cv) and close association of mitochondria with lipid sphere. 28,000X. Age, EC+ 46±1 1/2 hr. Basal lamina, bm; hemidesmosome, he; lipid sphere, li.

TABLES


li
li
bm
bm

Fig. 29. Spruce budworm. The organization of the microvilli and the development of smooth tubular endoplasmic reticulum (ster) in the apical region of an adult gland cell. The core (co) of the microvilli appears to continue within the cell as smooth endoplasmic reticulum (open arrows). 35,450X. Age, EC+ 46±1 1/2 hr. Coated vesicle, cv; endocuticle, en; microbodies, mb.

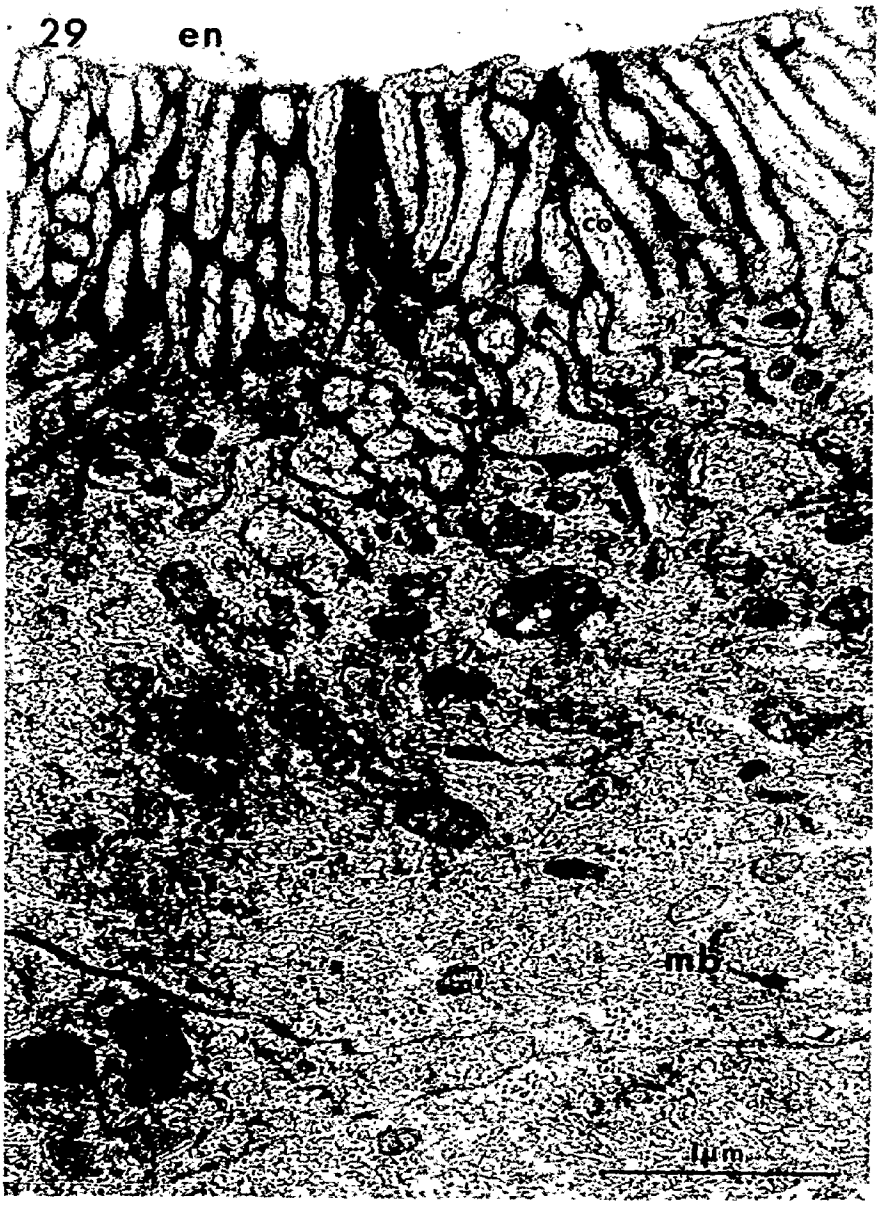

29
en
co
mb
1µm

Fig. 30. Spruce budworm. Microbodies near the lateral cell membranes in the apical region of the adult gland cell. The open arrow marks the slight density present in some microbodies. The black arrows indicate cisternae of endoplasmic reticulum along the lateral cell membranes. 55,800X. Age EC+ 46± 1 1/2 hr. Intercellular space, is; microbody, mb.

Fig. 31. Spruce budworm. Microbodies and their association with smooth (ster) and rough (rer) endoplasmic reticulum. 103,900X. Age, EC+ 31±7 hr. Mitochondrion, m; microbody, mb.

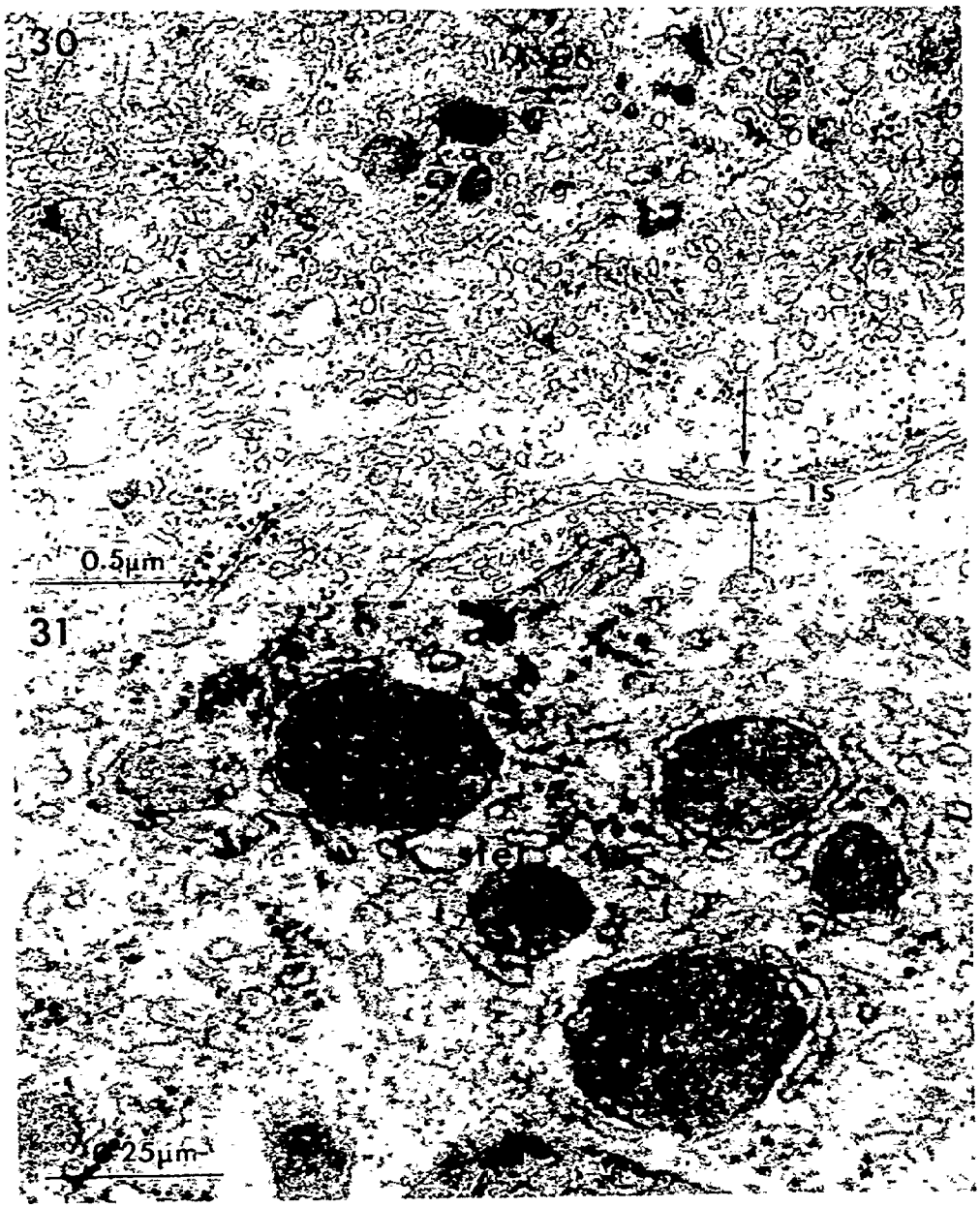

30
1s
0.5µm
31
1e
0.25µm

Fig. 32. Spruce budworm. The smooth endoplasmic reticulum penetrates the microvilli for almost their entire length. Throughout the length, microfibrils stretch from the 'core' to the lateral membranes. 170,900X. Age, EC+ 46±1 1/2 hr. 'Core', co; microfibrils, mf.

Fig. 33. Spruce budworm. Microvilli in transverse section illustrating the uniformity in size, the presence of the 'core', and the microfibrils stretching from the 'core' to the lateral membranes. 170,900X. Age, EC+ 46±1 1/2 hr. 'Core', co; microfibrils, mf.

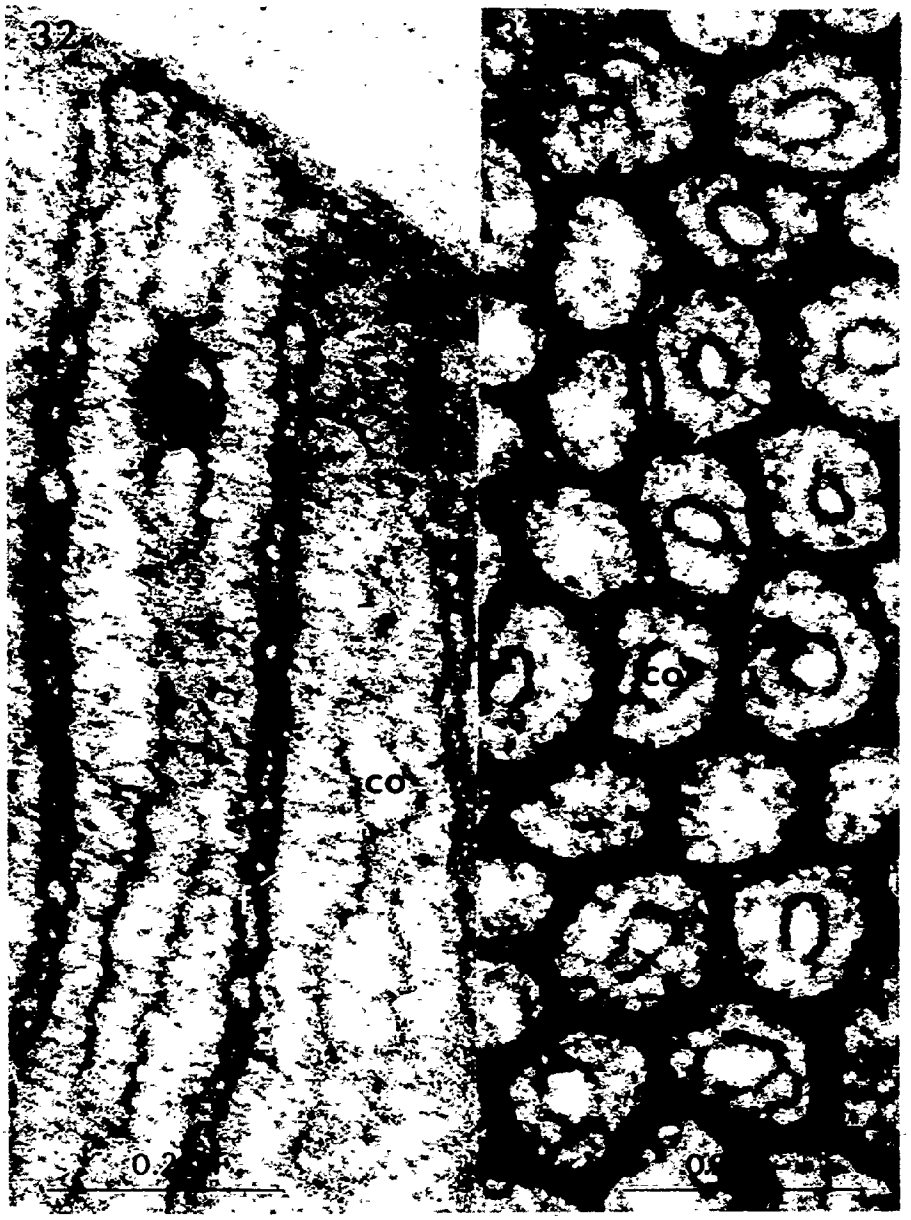

32
CO
O 2
CO
O2

Fig. 34. Spruce budworm. External layers of adult gland cuticle. The epicuticular filaments form a compact layer immediately beneath the dense epicuticle. The open arrows indicate pores in the inner cuticulin. 120,500X. Age, EC+ 120±7 hr. Inner cuticulin, ic; dense epicuticle, de; endocuticle, en; epicuticular filaments, ef; outer cuticulin, oc.

Insert: A group of epicuticular filaments terminating at an oval depression in adult gland cuticle. Fixed only in osmium tetroxide, not stained. 85,200X. Age, EC+ 36±9 hr. Dense epicuticle, de; oval depression, od.


34
de
0.2µm
od
de
ef
ic
en
0.2µm
oc

Fig. 35. Spruce budworm.

(a) The interpretation of the spatial relationship of organelles in an adult gland cell and the structure of the cuticle. Basal involution, bi; basal lamina, bm; coated vesicle, cv; cuticulin, cu; endocuticle, en; glycogen deposit, gd; Golgi complex, G; intercellular space, is; junctional areas, j; lipid sphere, li; microbody, mb; microtubule, mt; microvilli, mv; mitochondria, m; multivesicular body, mvb; nucleus, n; pore, p; tracheole, tr.

(b) The termination of the epicuticular filaments and the outer layers of the cuticle. Inner cuticulin, ic; dense epicuticle, de; epicuticular filaments, ef; outer cuticulin, oc; oval depression, od; pore, p.

(c) Diagrammatic interpretation of the structure of the microvilli and the contents of a pore canal. Within the pore canal, the filamentous structure is found only next to the microvilli where the microfibrils are not helicoidally arranged. The tubular epicuticular filaments are present in all lamellae of the endocuticle. 'Core' of microvilli, co; endocuticle, en; epicuticular filaments, ef; microvilli, mv; pore canal, pc.

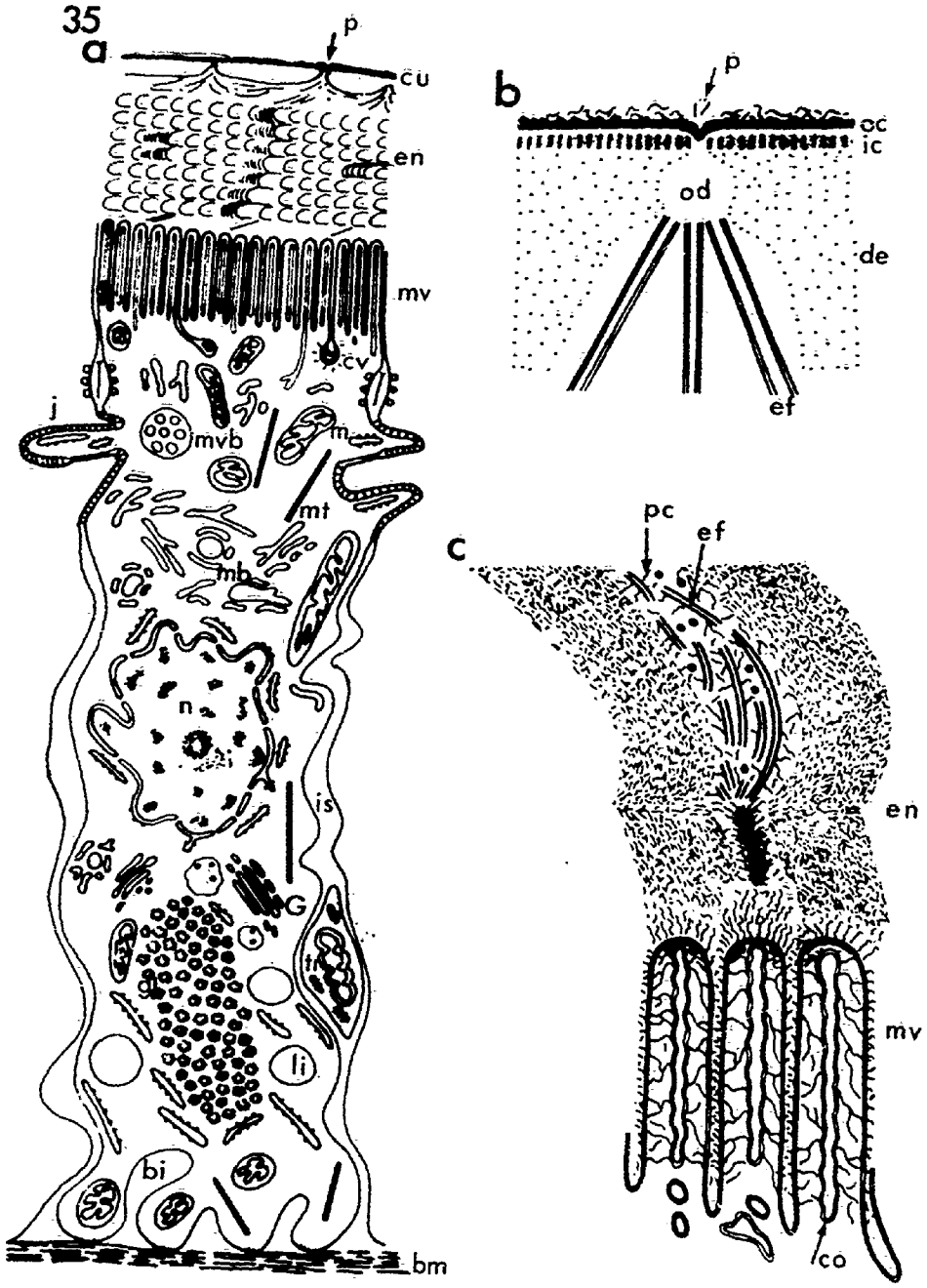

35
a
p
cu
en
mv
cv
j
mvb
m
mt
mb
n
i
is
G
tf
li
bi
bm
b
p
oc
ic
od
de
ef
c
pc
ef
en
mv
co

Fig. 36. Tussock moth. Adult gland cells showing extensive development of smooth cisternal endoplasmic reticulum (scer). 13,750X. Age, EC+ 72±3 hr. Cuticular extension, ce; fenestrae of cisternae (arrow); microbody, mb; microvilli, mv; mitochondrion, m.

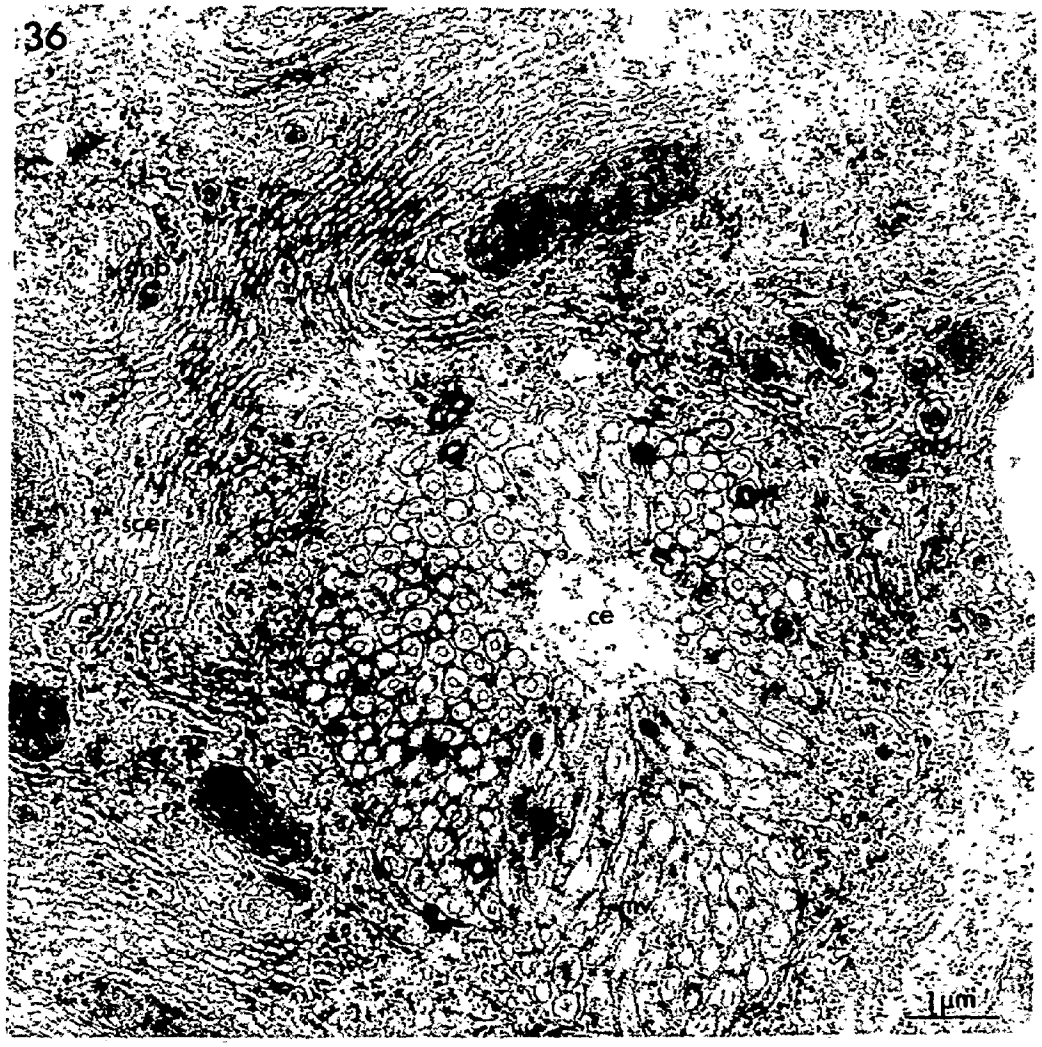
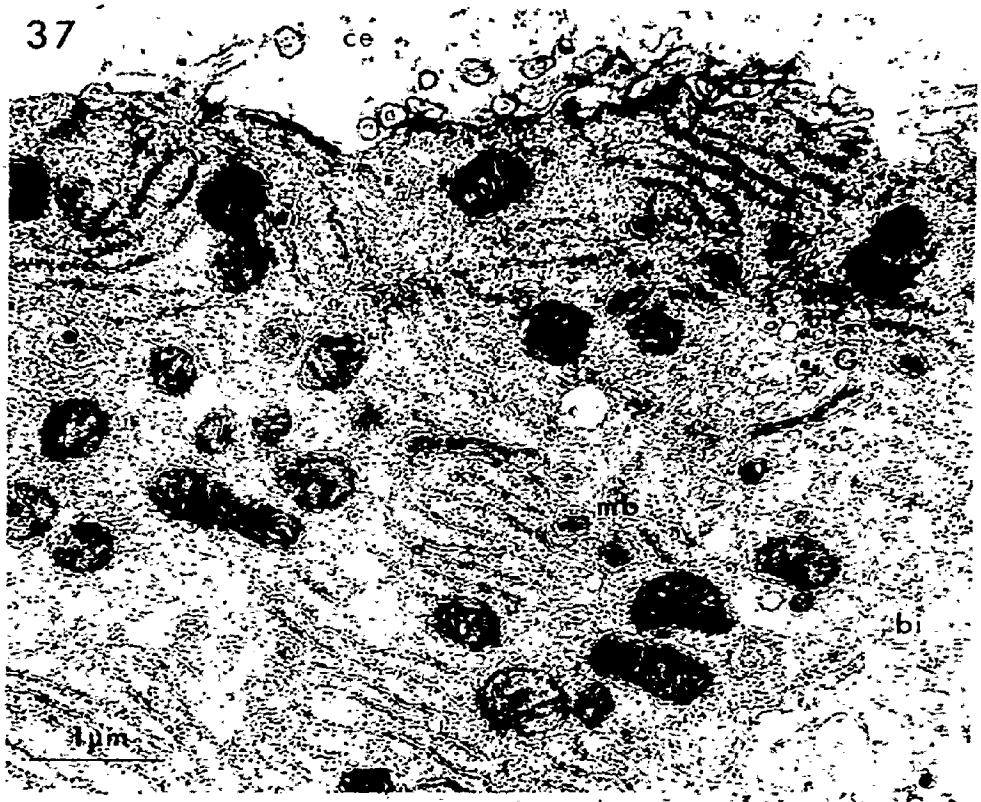

36
mb
sce
ce
mv
1µm

Fig. 37. Tussock moth. Adult gland cell showing well-organized rough endoplasmic reticulum in a cell where there are few microvilli. 19,500X. Age, EC+ 1 day mated. Basal involution, bi; cuticular extension, ce; Golgi complex, G; mitochondrion, m; microbody, mb.

Fig. 38. Tussock moth. Adult gland cells showing well-organized smooth cisternal endoplasmic reticulum in a cell where there are few microvilli. Note plasma membrane of adjacent cell (adj) is microvillate. 25,000X. Age, EC+ 72±3 hr. Cuticular extension, ce. The arrow indicates fenestrae of cisternae.

37



38

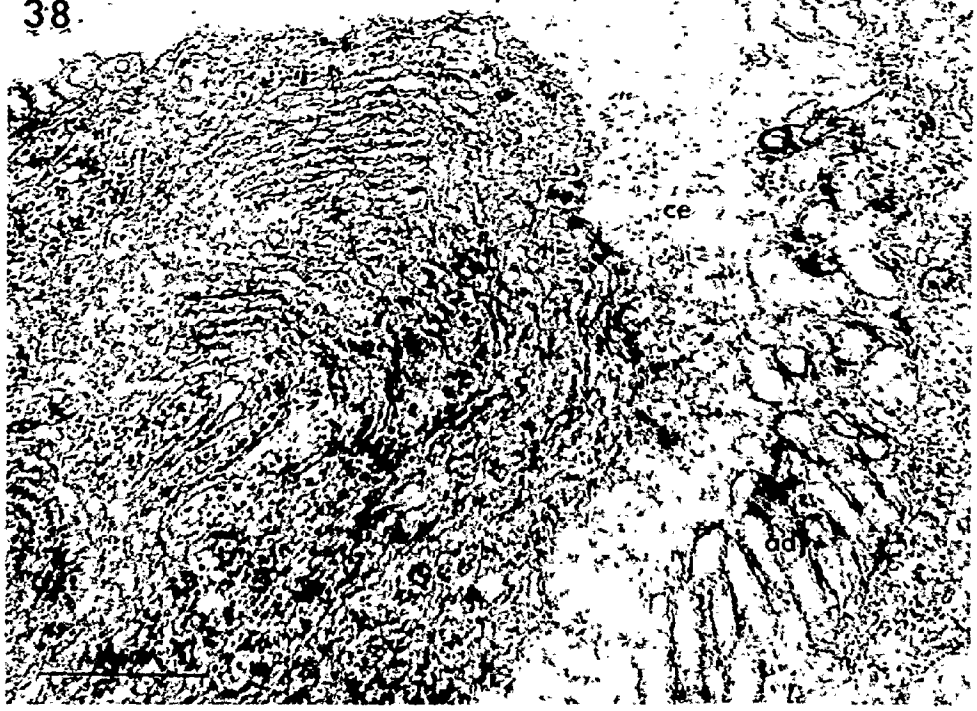


Fig. 39. Tussock moth. Membranous structure located near nucleus in adult gland cell. The area outlined in open rectangle is illustrated in Fig. 40 at a higher magnification. 5,600X. Age EC+ 1 day mated. Basal involution, bi; basal lamina, bm; Golgi complex, G; membranous organelle, mo; microvilli, mv; nucleus, n.

Fig. 40. Tussock moth. Higher magnification of left tip of structure shown in Fig. 39. 21,000X. Age, EC+ 1 day mated. Golgi complex coated vesicles, Gv; microbodies, mb; mitochondrion, m; nucleus, n.

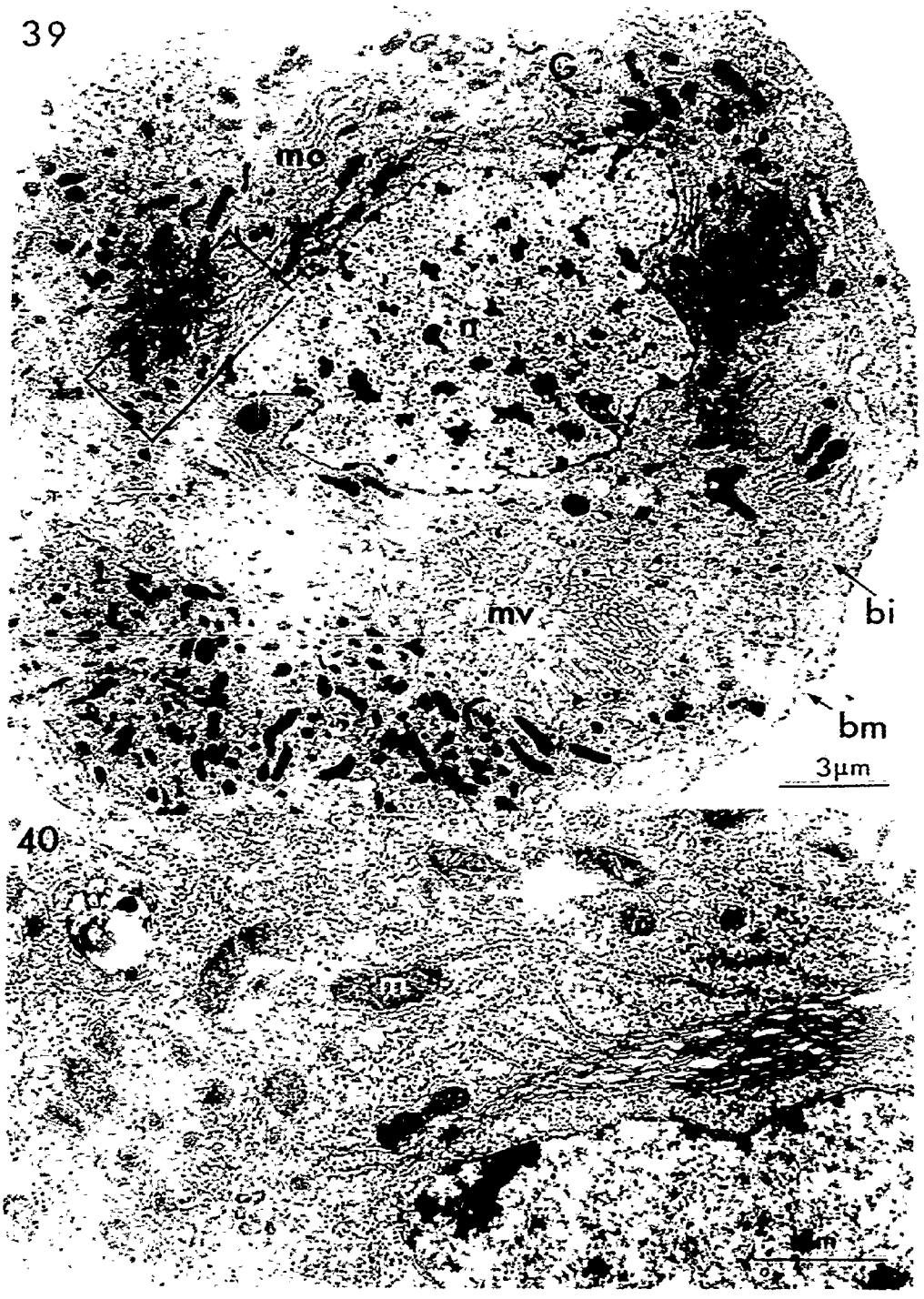

39
me
n
mv
bi
bm
3µm
40
n

Fig. 41. Tussock moth. Outer layers of cuticle from the level at which the lamellate endocuticle appears. 40,500X. Age, EC+ 72±3 hr. Dense epicuticle, de; epicuticular filaments, ef; lamellate endocuticle, en; inner cuticulin, ic; outer cuticulin, oc; oval depression, od; pore canal, p; outer uneven layer, ue.

Fig. 42. Tussock moth. Smooth cisternal endoplasmic reticulum at bases of microvilli. Arrow indicates tubule of smooth endoplasmic reticulum extending towards microvilli. 54,250X. Age, EC+ 72±3 hr. Cuticular extension, ce; nucleus, n.

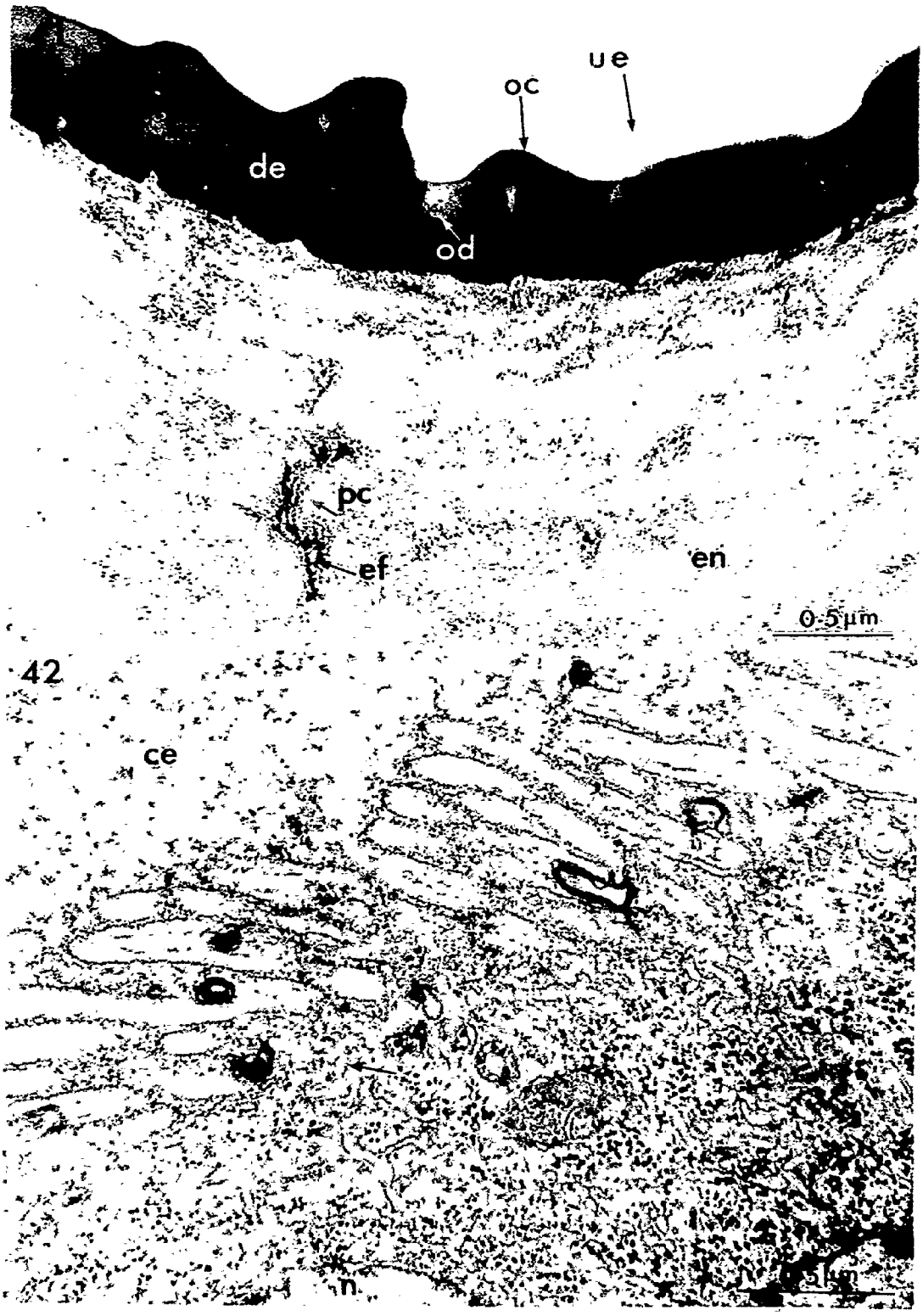

ue
oc
de
od
pc
ef
en
0·5 µm
42
ce
n

Fig. 43. Tussock moth. The interpretation of the spatial relationship of the organelles to each other within an adult gland cell and the relationship of the cell to the overlying cuticle. Basal involution, bi; basal lamina, bm; dense epicuticle, de; epicuticular filaments, ef; endocuticle, en; endocuticular projection, ce; Golgi complex, G; inner cuticulin, ic; cell junctions, j; microbodies, mb; membranous structure, mo; microvilli, mv; outer cuticulin, oc; oval depression, od; pore canal, pc; surface view of smooth cisternal endoplasmic reticulum, scer; uneven layer, ue.

43

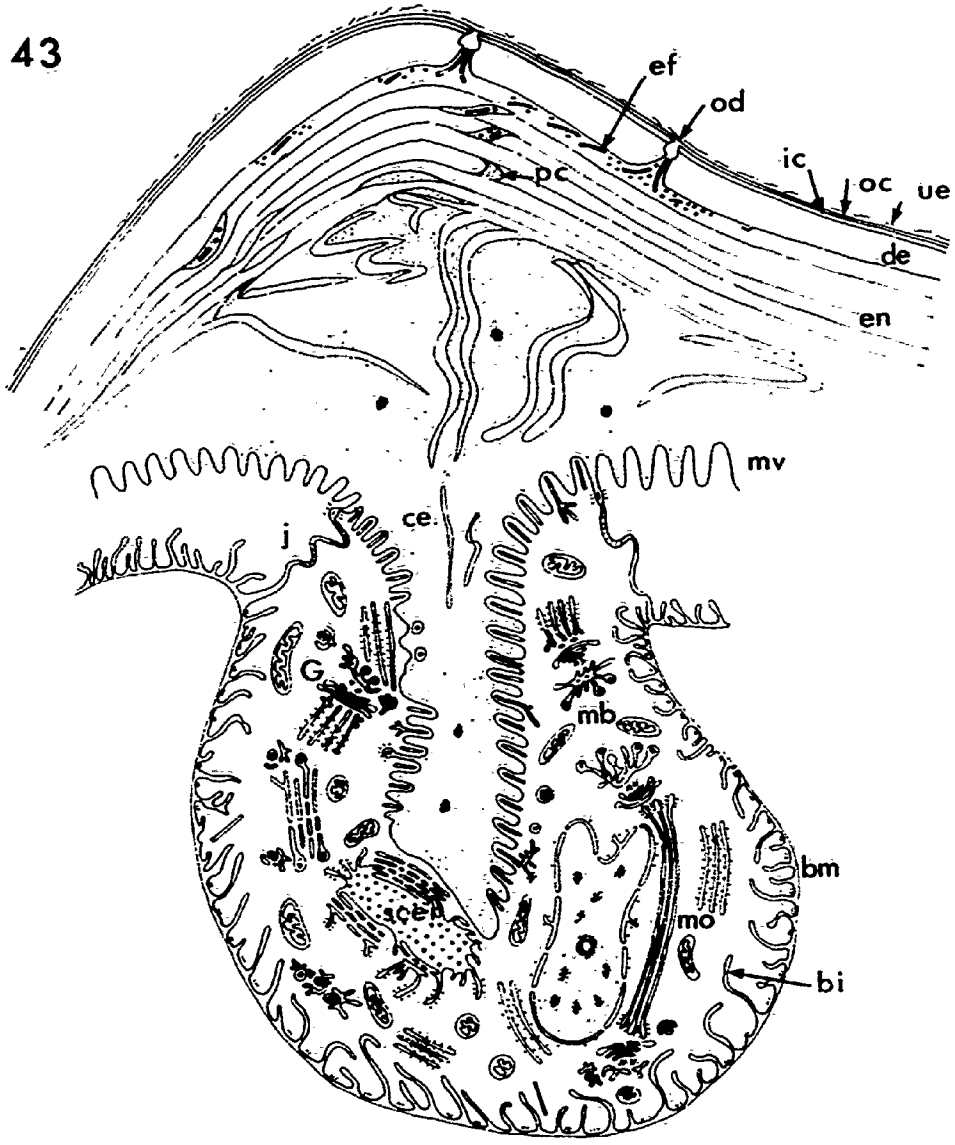


Fig. 44. Cabbage looper. Bilayered appearance of basal lamina membrane under adult gland cells. (Arrows indicate tubules from b2 which pass singly or in small groups through b1 and are re-organized next to basal plasma membrane as in Fig. 45). 52,500X. Age EC+7 hr. Basal involutions, bi; hemidesmosomes, he; gaps between layers 1 and 2 of basal lamina, ga; reorganized portion of layer 2 within basal involutions, ro; termination of reorganized portion, te. Layers 1 and 2 of basal lamina, b1, b2.

Fig. 45. Cabbage looper. Reorganized portion (ro) of layer 2 of basal lamina as it appears immediately beneath basal plasma membrane. 10,000X. Age, EC+4 hr. Coated vesicle, cv; hemidesmosome, he; microbodies, mb; reorganized portion of layer within basal involution, ro; termination of reorganized portion, te; layers 1 and 2 of basal lamina, b1, b2.


44
bi
te
bl
he
b2
ga
0.5 µm
45
te
bl
ro
ro
b2
0.5 µm

Fig. 46. Cabbage looper. Termination (te) of reorganized portion of layer 2 within basal involution showing associated coated vesicles (cv) and hemidesmosomes (he). 76,250X. Age, EC+19 hr. Mitochondrion, m; rough endoplasmic reticulum, rer.

Fig. 47. Cabbage looper. Numerous coated vesicles observed near basal plasma membrane of young adults. 73,000X. Age, EC+7 hr. Basal involution, bi; coated vesicles, cv; termination of reorganized portion of layer 2, te; layer 1 of basal lamina, l.


46
m
GV
CV
rer
te
he
0.3µm
47
bi
te
CV
l
0.3µm

Fig. 48. Cabbage looper. Appearance of basal lamina in vicinity of tracheal penetration between gland cells. 17,800X. Age, 36 hr pre-eclosion. Granular haemocyte, gh; gland cell, gc; reorganized portion of layer 2 between haemocyte and gland cell, ro; trachea, tr. Layers 1 and 2 of basal lamina, b1, b2.


4B
gc
gh
b2
b1
ro
gh
tr
b1
ro
1µm

Fig. 49. Cabbage looper. Lipid sphere near apical portion of adult gland cell. 22,500X. Age, EC+1 hr. Endocuticle, en; lipid sphere, li; mitochondrion, m; microbody, mb; rough endoplasmic reticulum, rer. Arrow indicates tubule of smooth endoplasmic reticulum within microvilli.

Fig. 50. Cabbage looper. Lipid spheres near base of gland cell, some of which appear free in cytoplasm not surrounded by microbodies. 15,000X. Age, EC+36 hr. Basal lamina, b; Golgi complex, G; lipid, li; mitochondria, m; nucleus, n; rough endoplasmic reticulum, rer.


49
fer
en
mb
m
1 µm
50
n
G
r.er
b
1 µm

Fig. 51. Cabbage looper. Gland cell from young adult in which smooth tubular endoplasmic reticulum (ster) and patches of smooth cisternal endoplasmic reticulum (scer) are appearing. 133,000X. Age, EC+1 hr. Golgi complex, G; mitochondria, m; microbodies, mb; rough endoplasmic reticulum, rer.

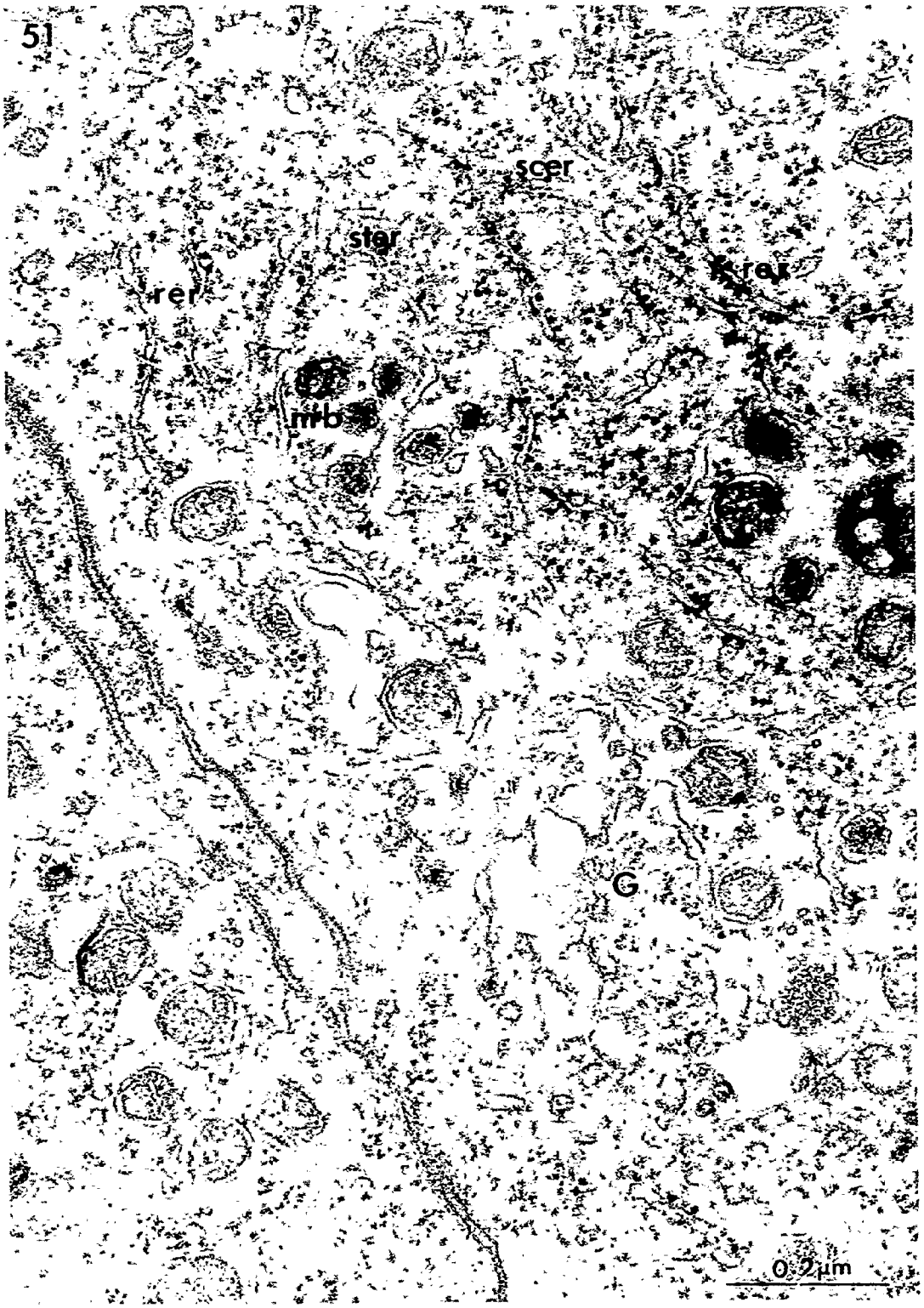

51
sER
sER
rER
rER
mb
G
0.2 µm

Fig. 52. Cabbage looper. Organization of cytoplasmic organelles in vicinity of a lipid sphere (li) located midway in a gland cell. Note close proximity of a Golgi complex (G) and the elongate, as well as oval, appearance of the microbodies (mb). The microbodies shaped like dumb-bells may indicate folded elongate microbodies but most probably show their origin as distensions of smooth tubular endoplasmic reticulum. 104,150X. Age, EC+4 hr. Mitochondrion, m; smooth endoplasmic reticulum, ser.

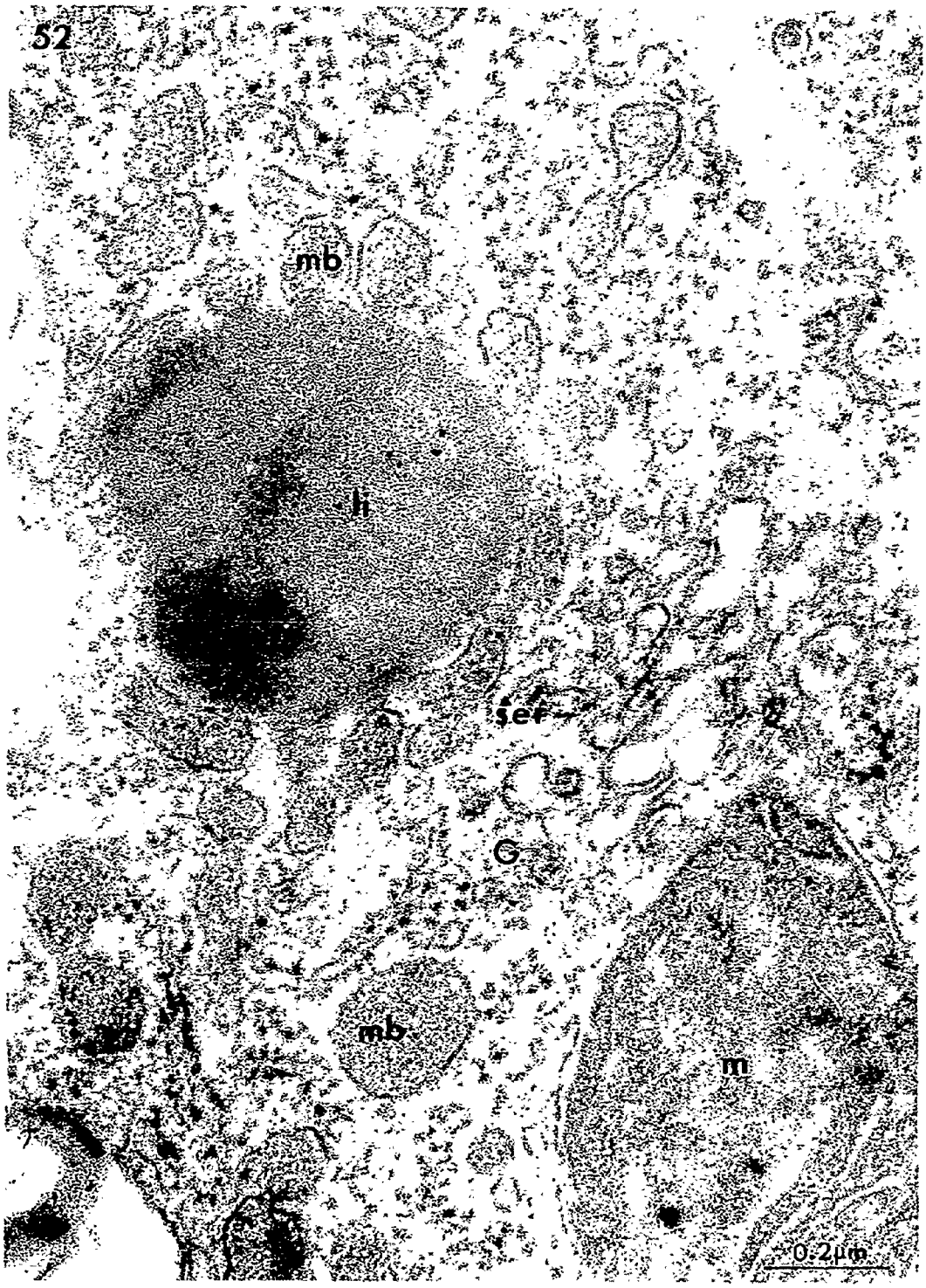
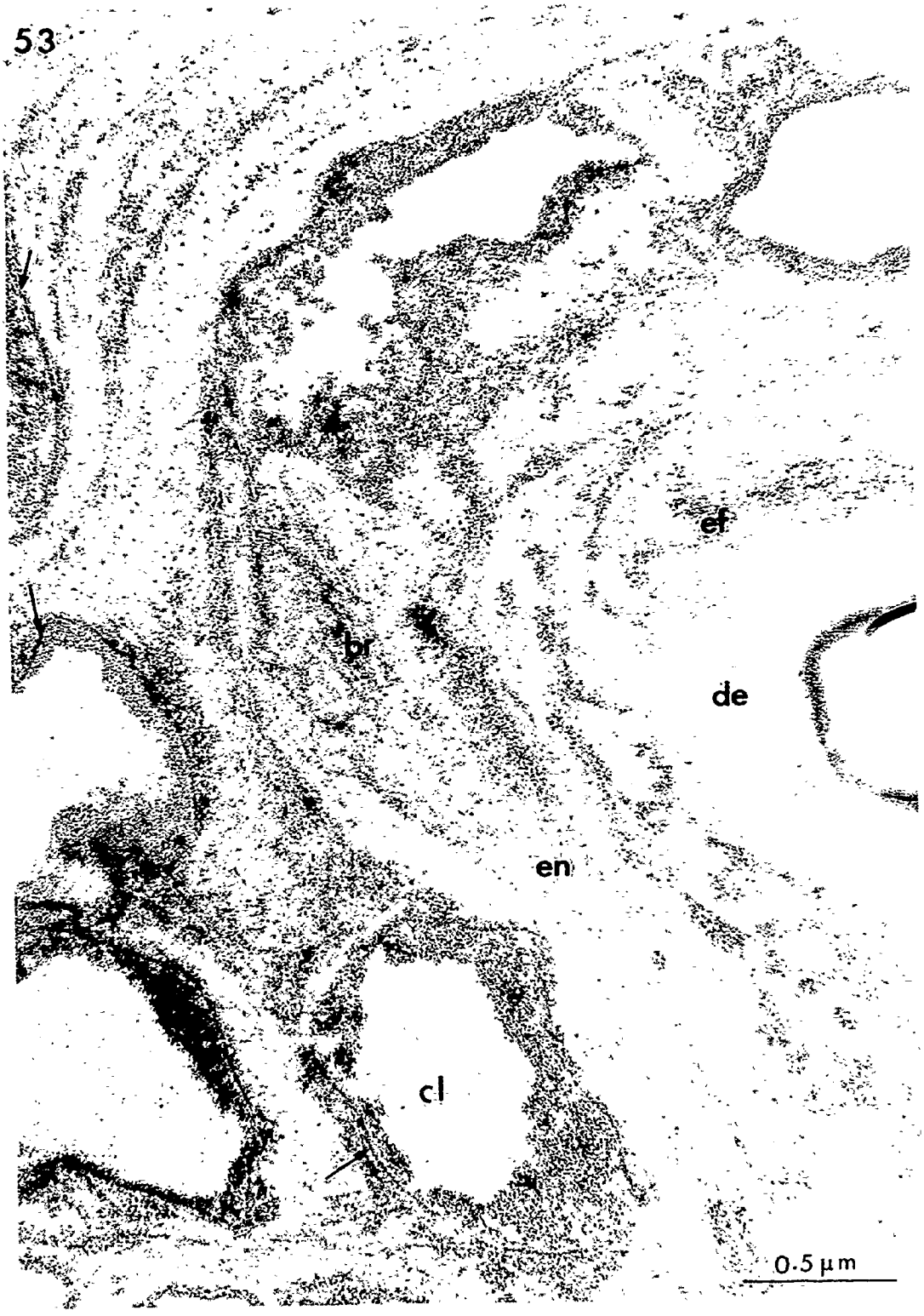

52
mb
h
ser
G
mb
m
0·2µm

Fig. 53. Cabbage looper. Lipid deposits (cl) within adult gland cuticle. Note that a membrane is obvious around some lipid deposits (arrows). 50,850X. Age, EC+91 hr. Branch of lipid, br; dense epicuticle, de; epicuticular filaments, ef; endocuticle, en.


53
ef
br
de
en
cl
0.5 µm

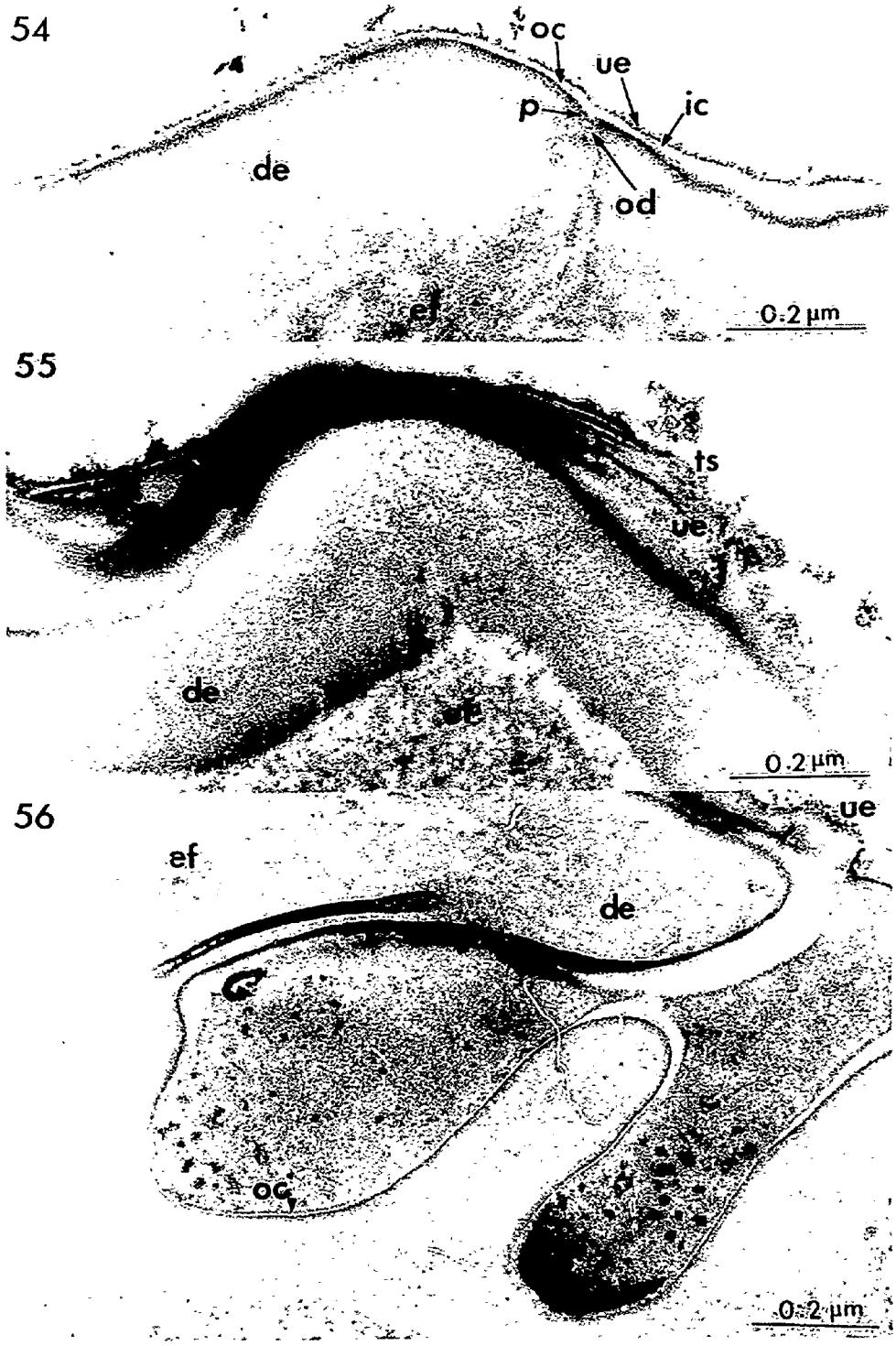
Figs. 54-56. Cabbage looper. Different structures observed in and on outer layers of cuticle overlying gland cells.

Fig. 54. A thin uneven layer (ue) separated by a small distance from outer cuticulin (oc). 117,000X. Age, EC+1 hr.

Fig. 55. Tubular structures (ts) sometimes observed intermingling with outer uneven layer (ue). 117,000X. Age, EC+3 hr.

Fig. 56. Usual appearance of cuticle in vicinity of such uneven layer (ue) and outer cuticulin (oc). 104,250X. Age, EC+19 hr.

Dense epicuticle, de; epicuticular filaments, ef; inner cuticulin, ic; oval depression, od; pore, p.


54
oc
ue
ic
p
de
od
ef
0.2 µm
55
ts
ue
p
de
ef
0.2 µm
56
ue
ef
de
oc
0.2 µm

Figs. 57-59. Stages in development of lipid spheres in gland cells from young adults.

Fig. 57. Golgi complex before many lipid spheres are free in cytoplasm. 38,150X. Age, EC+5 hr. Golgi complex, G; microbodies, mb; smooth endoplasmic reticulum near maturing face of Golgi complex, ser.

Fig. 58. Glancing surface section through cap of smooth cisternal endoplasmic reticulum (arrow) and attached microbodies (mb). 46,150X. Age, EC+4 hr.

Fig. 59. Section believed to represent cross section through smooth endoplasmic reticulum (arrow) forming 'cap' on lipid sphere (li). 50,500X. Age, EC+5 hr.


57
Hb
58
59
li

Fig. 60. Cabbage looper. Spatial relationship between Golgi complex (G); cap of smooth endoplasmic reticulum (s) and microbodies (mb) in gland cell of young adult. 140,600X, Age, EC+ 1 hr. Mitochondrion (m).

Fig. 61. Cabbage looper, Elongate microbody (mb) near developing lipid sphere 106,000X Age, EC+ 1 hr.

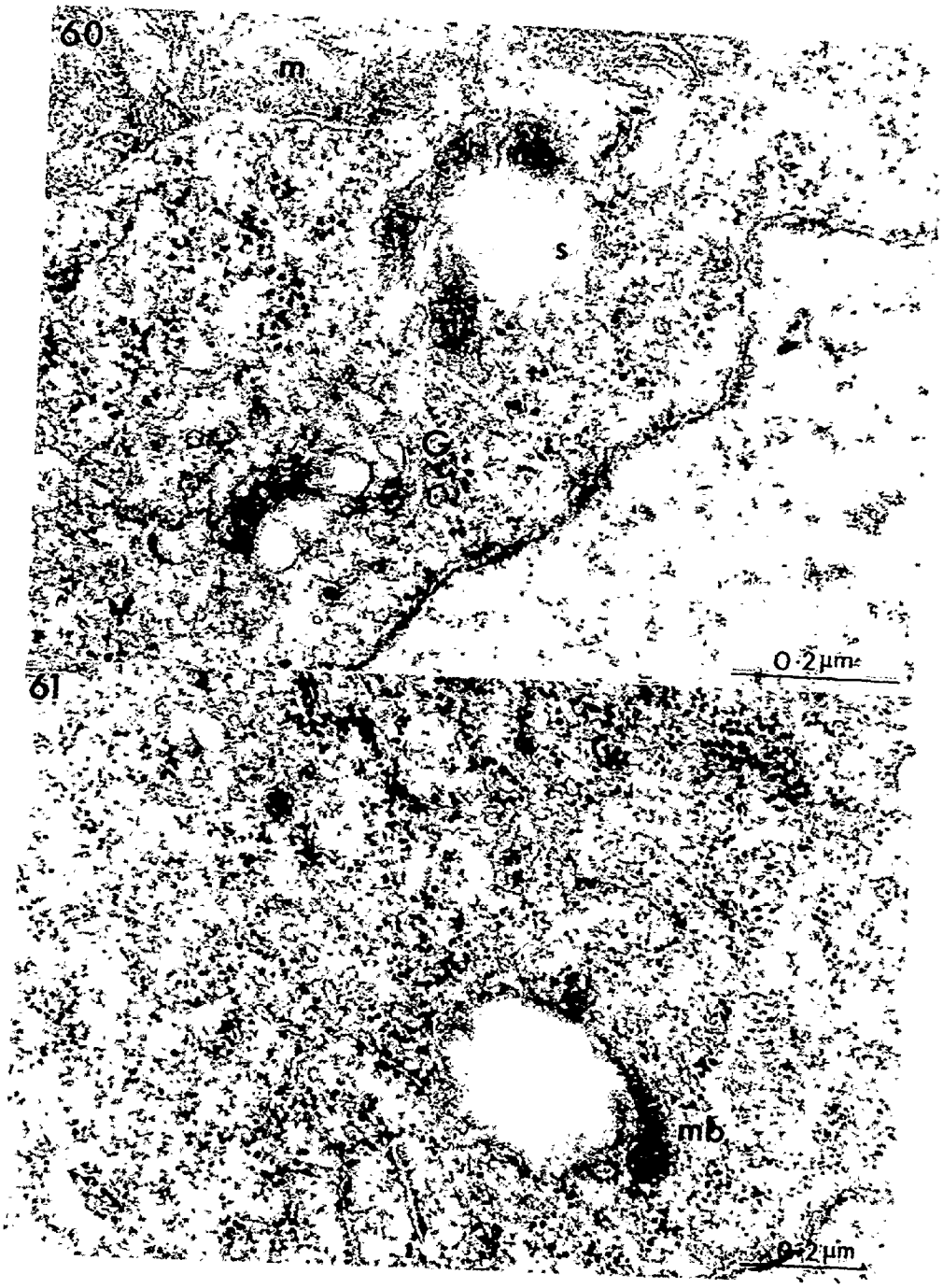

60
m
s
G
61
0·2 µm
mb
0·2 µm

Fig. 62. Cabbage looper. Appearance of lipid sphere and associated microbodies as most frequently observed in cytoplasm of adult cells. 66,000X. Age, EC+7 hr. Lipid, li; mitochondrion, m; microbodies, mb; smooth endoplasmic reticulum, ser.

62

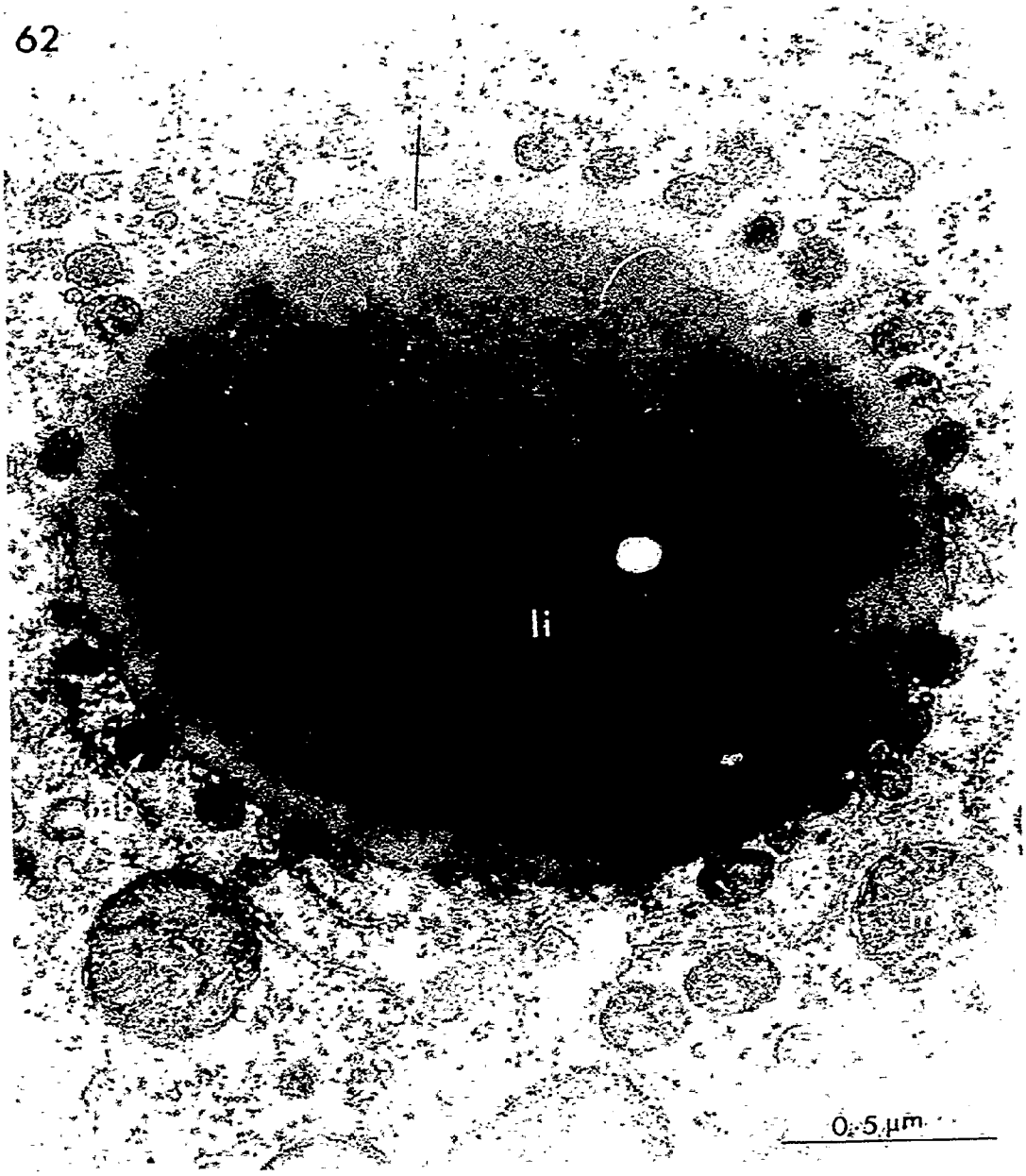


Fig. 63. Cabbage looper. Lipid sphere at apical portion of cell in process of pushing out plasma membrane between microvilli. Note lack of associated microbodies as compared to Fig. 62. 128,750X. Age, EC+ 4 days. Lipid, li; microbody, mb; microvilli, mv.

63

Fig. 64. Cabbage looper. Lipid spheres still within confines of gland cells and extending into cuticle. This process for a time results in the formation of unusual microvilli with bulbous tips - each bulb containing a lipid sphere. 66,550X. Age, EC+ 24 hr. Endocuticle, en; lipid spheres, li; microvilli, mv; smooth endoplasmic reticulum initially forming 'core' of microvilli; co; pore canal, pc

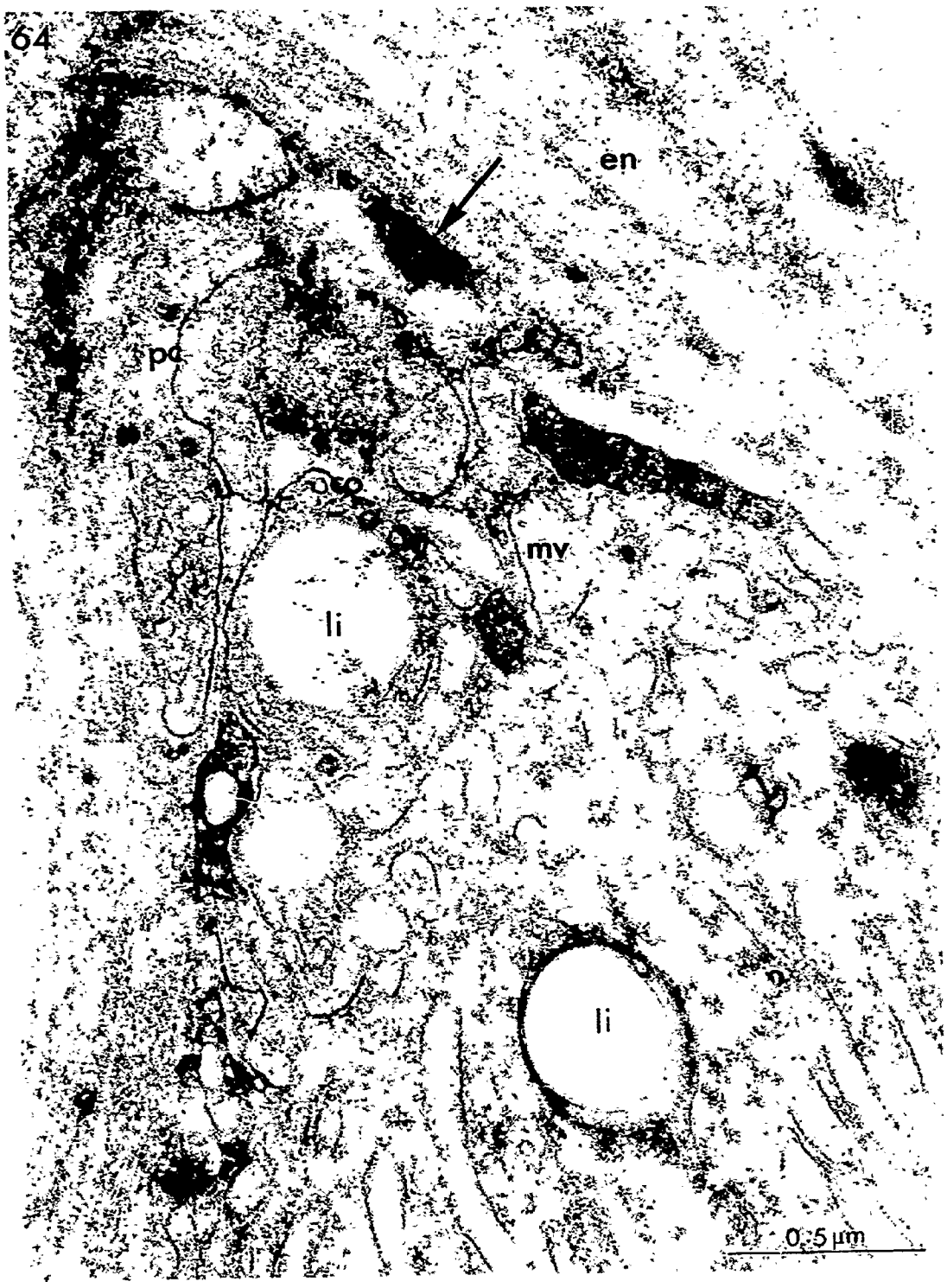

64
en
pc
co
my
li
li
0,5 µm

Fig. 65. Cabbage looper. Lipid spheres as they appear within cuticle but still within confines of cell membrane (broken arrows). 88,400X. Age, EC+ 91 hr. cuticular lipid cl; endocuticle, en; microvilli, mv.

Fig. 66. Cabbage looper. Branching (br) of lipid deposits within cuticle but still within confines of cell membrane (broken arrows). 88,400X. Age, EC+ 91 hr. cuticular deposit, cl; endocuticle, en; microvilli, mv.

Solid arrows - believed to represent appearance of area within cell after most of the lipid which it has contained has moved into cuticle. See also arrows Figs 64, 67.


65
cl
er
mv
0.5 µm
66
mx
cl
0.5 µm

Fig. 67. Cabbage looper. Outer layers of cuticle over adult gland cells. The large deposit of cuticular lipid (cl) and its branches (br) are surrounded by a membrane (broken arrows). The smaller clear area (asterisk) which represents cuticular lipid not surrounded by a membrane. Near the area marked with an asterisk there are tubules ('lipid' tubules, white arrows, lt) which contain a dense osmiophilic core and are thus structurally distinct from the epicuticular filaments (black arrows, ef). 81,000X. Age, EC+ 91 hr. Dense epicuticle, de; endocuticle, en.


de
ll
ef
ef
br
en
cl
0.3µm

Fig. 68. Cabbage looper. 'Lipid' tubules (white arrows, lt) within and outside cuticle over adult gland cells. 80,000X. Age, EC+ 3 hr. Dense epicuticle, de; epicuticular filaments, ef; endocuticle, en; 'lipid' tubules, lt; oval depression, od; pore, p.


68
de
lt
od
p
ef
lt
en
0·3 µm

Fig. 69. Cabbage looper. Structures observed in haemocytes, gland cells and cuticle as related to formation and transport of lipid and compared with the age of the insect.

1. Granular haemocytes release material which participates in the formation of the basal lamina.
2. Some of this material is taken up by the cell and is converted to gland cell lipid.
3. Lipid accumulates as it moves towards the apex of the cell.
4. The accumulation of lipid ceases near the base of the microvilli and protrudes into the cuticle.
5. The lipid, remaining within the confines of the cell membrane, moves into the cuticle and is stored there.
6. A tubular form of the lipid passes to the outside of the gland and insect by pores in the inner cuticulin.

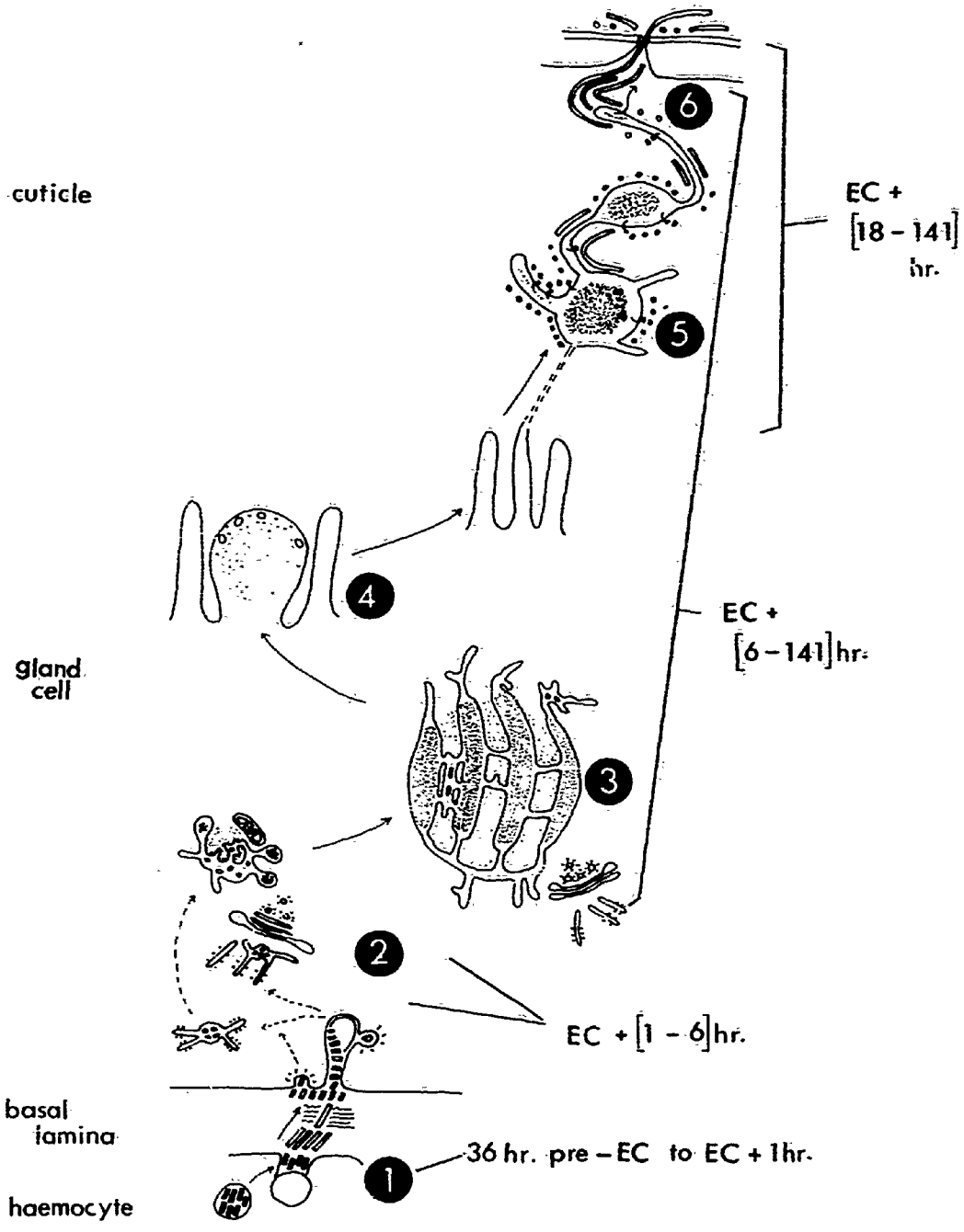

cuticle
6
EC +
[18 – 141]
hr.
5
4
EC +
[6 – 141]hr.
gland
cell
3
2
EC + [1 – 6]hr.
basal
lamina
1
36 hr. pre – EC to EC + 1hr.
haemocyte

Fig. 70. Cabbage looper. The interpretation of the spatial relationship of organelles in a gland cell and the structure of the cuticle from an adult insect older than 12 hours. Layer 1 of basal lamina, b 1; layer 2 of basal lamina, b 2; basal involution, bi; cuticular lipid, cl; coated vesicle, cv; epicuticle, e; endocuticle, en; filaments, f; Golgi complex, G; inner cuticulin, ic; intercellular space, is; junctions, j; lipid sphere, li; mitochondrion, m; microbody, mb; microvilli, mv; multivesicular body, mvb; outer cuticulin, oc; oval depression, od; pore canal, pc; reorganized portion of basal lamina within basal infolds, ro; termination of reorganized portion, te; uneven layer, ue.

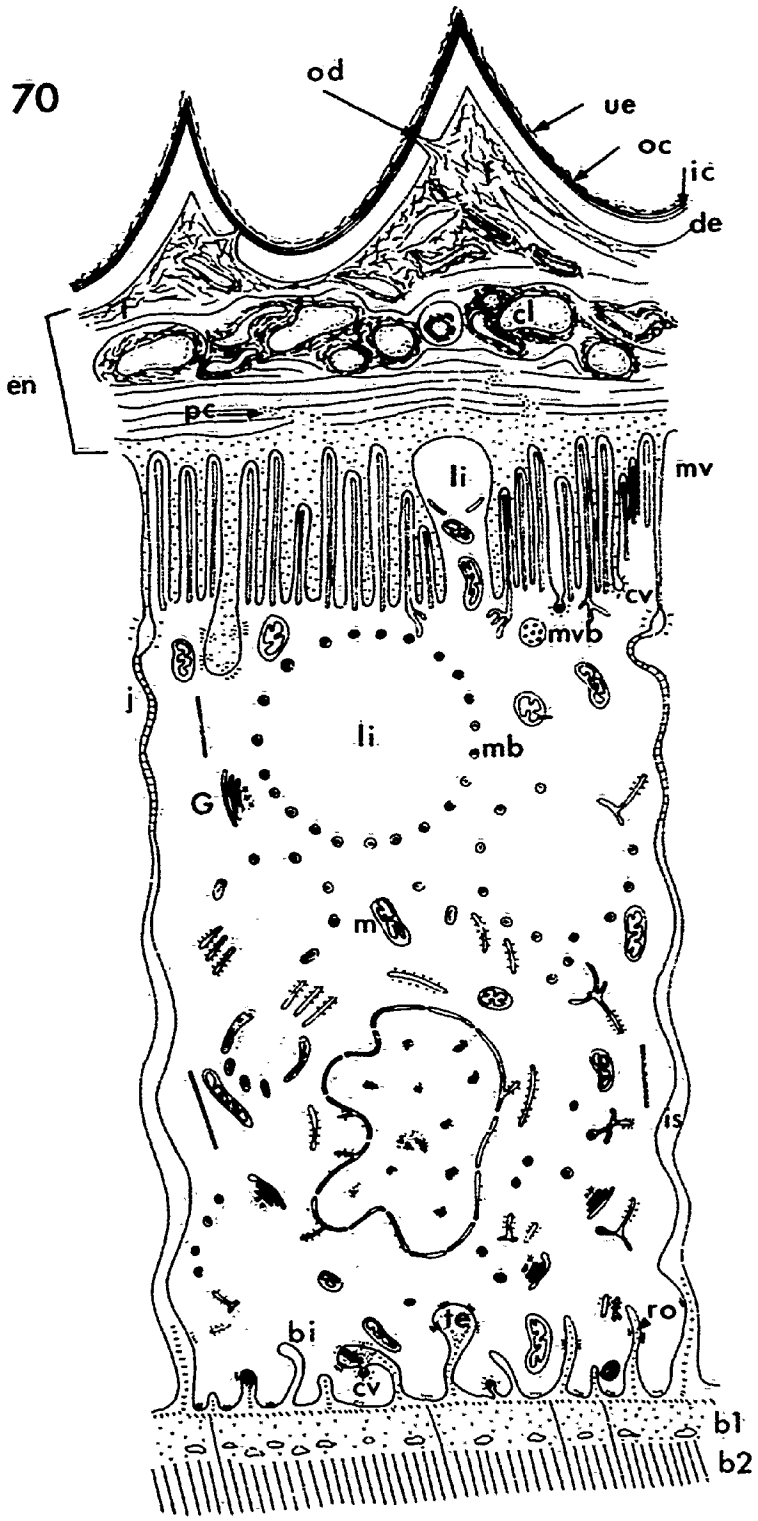

od
ue
oc
ic
de
cl
en
pe
li
mv
cv
mvb
j
li
mb
G
m
is
bi
is
ro
cv
b1
b2

Fig. 71. Cabbage looper. Modifications at lateral edges of gland to accommodate muscle attachment as exemplified by the cabbage looper. 3335X. Age, EC+36 hr. Cuticle, c; gland cell, gc; lipid sphere, li; nucleus, n. Layer 1 of basal lamina, b1; layer 2 of basal lamina, b2. Arrow indicates direction with respect to this section where muscle would attach as observed going from right to left in Fig. 71.

Fig. 72. Cabbage looper. Modifications of cells and cuticle at lateral edges of gland at site of muscle attachment. 6,100X. Age, EC+ 36 hr. Unmodified cuticle, c; cuticle modified for muscle attachment, cm; epidermal cells, ec; extensions of epidermal cells, ee; muscle, mc.

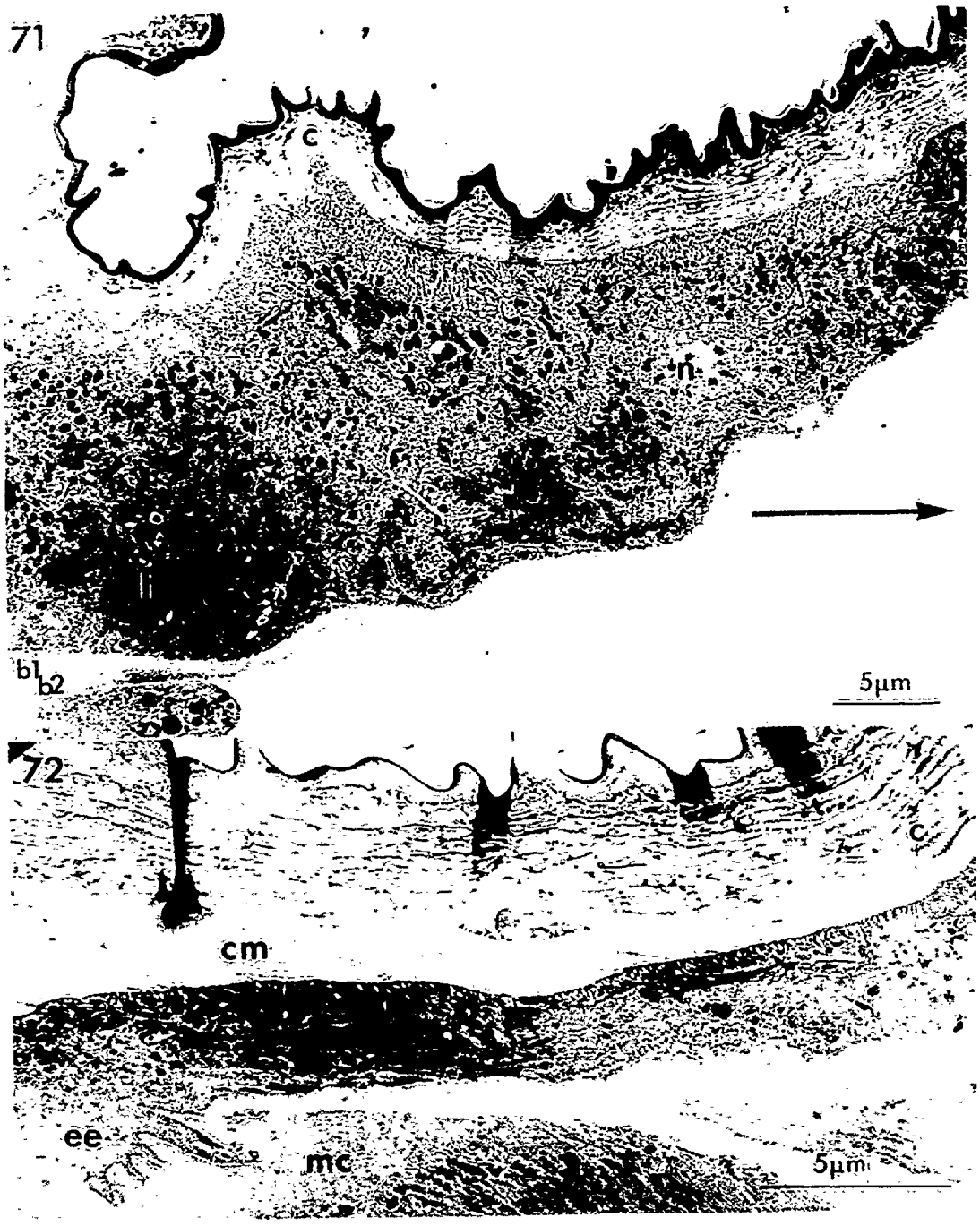
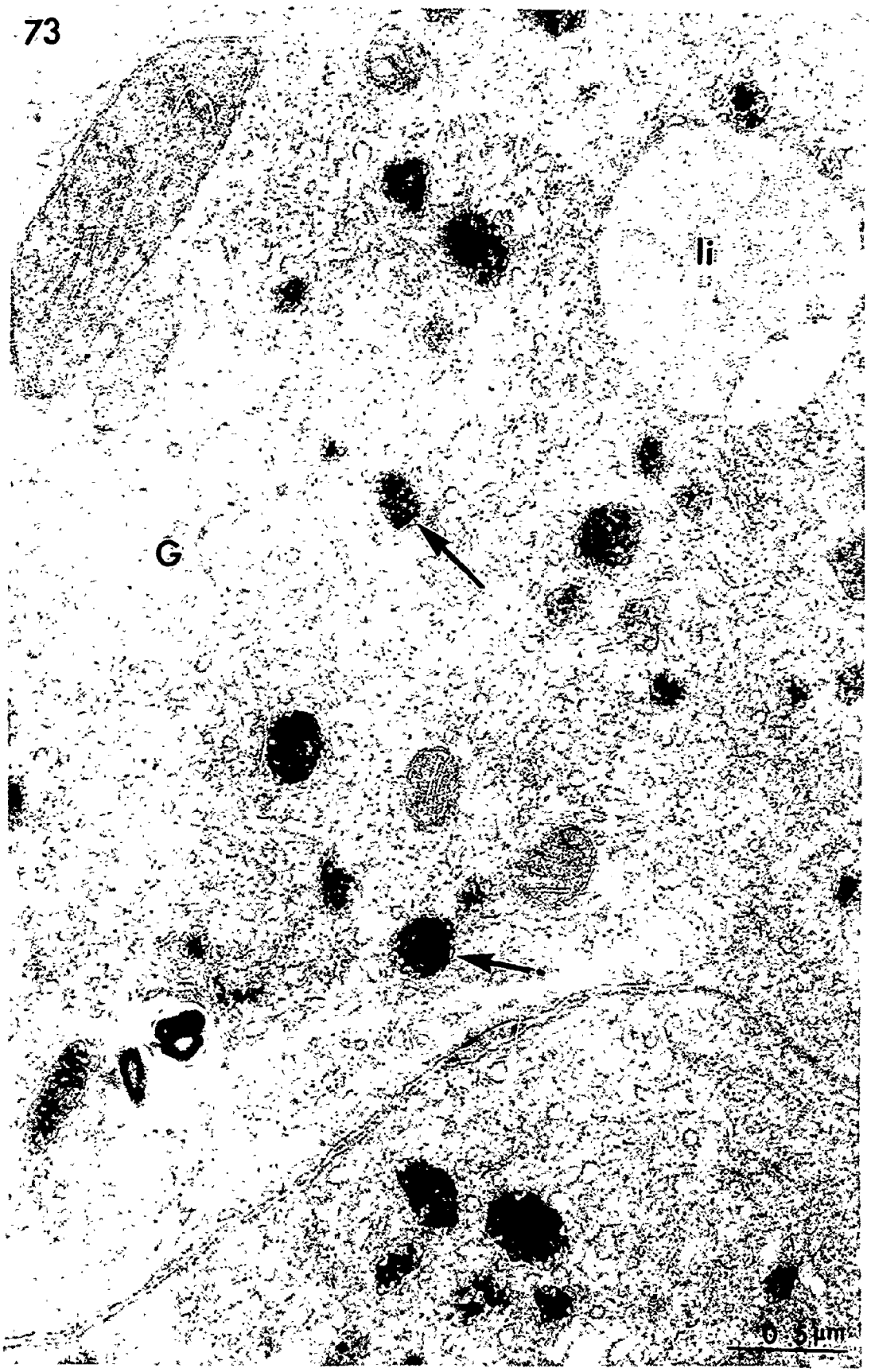

71
c
n
b1
b2
5µm
72
c
cm
ee
mc
5µm

Fig. 73. Spruce budworm. Positive reaction for catalase as exhibited by microbodies (arrows) when incubated in alkaline DAB medium containing hydrogen peroxide. 41,600X. Age, EC + 2 days. Golgi complex, G; lipid sphere, li.


73
li
G
0,5µm

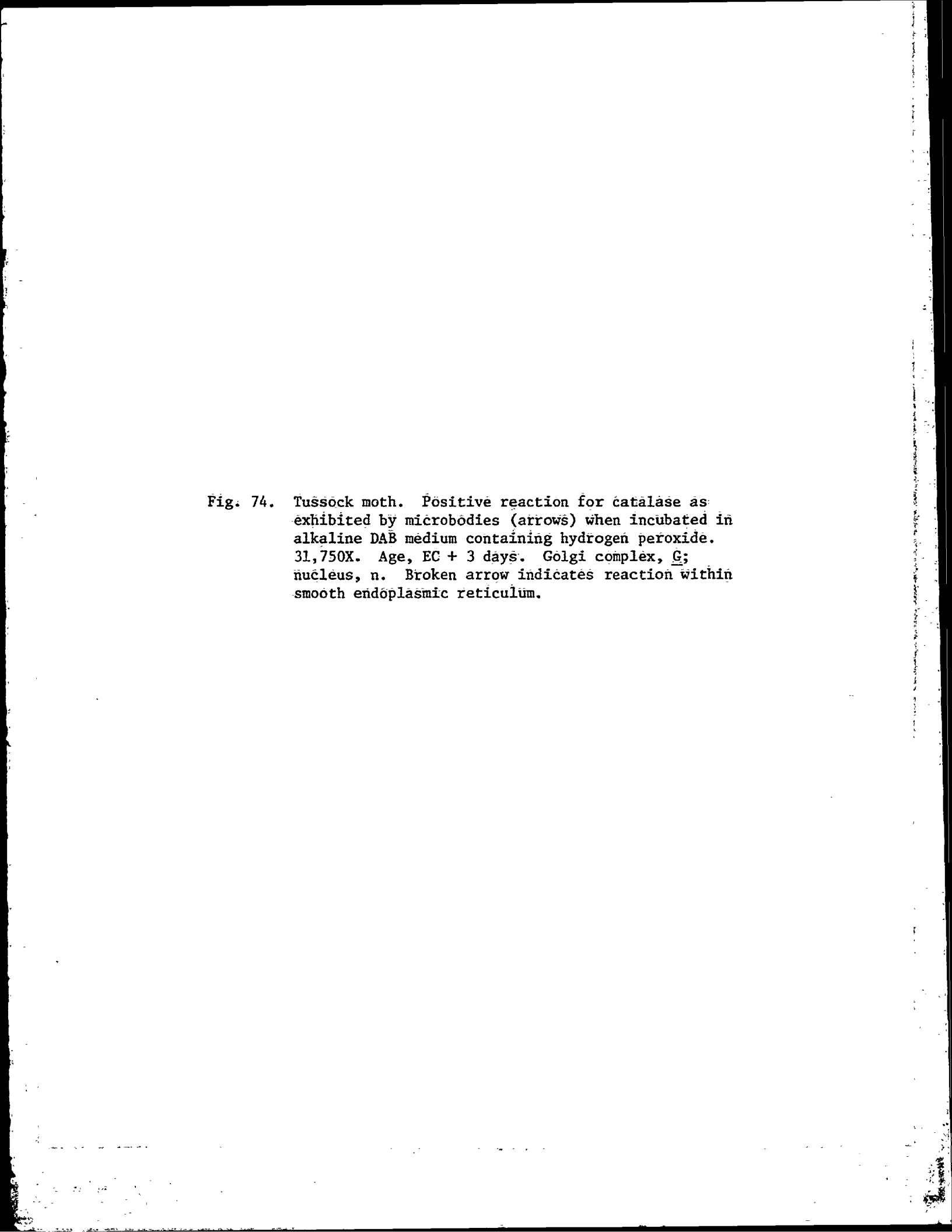


Fig. 74. Tussock moth. Positive reaction for catalase as exhibited by microbodies (arrows) when incubated in alkaline DAB medium containing hydrogen peroxide. 31,750X. Age, EC + 3 days. Golgi complex, G; nucleus, n. Broken arrow indicates reaction within smooth endoplasmic reticulum.

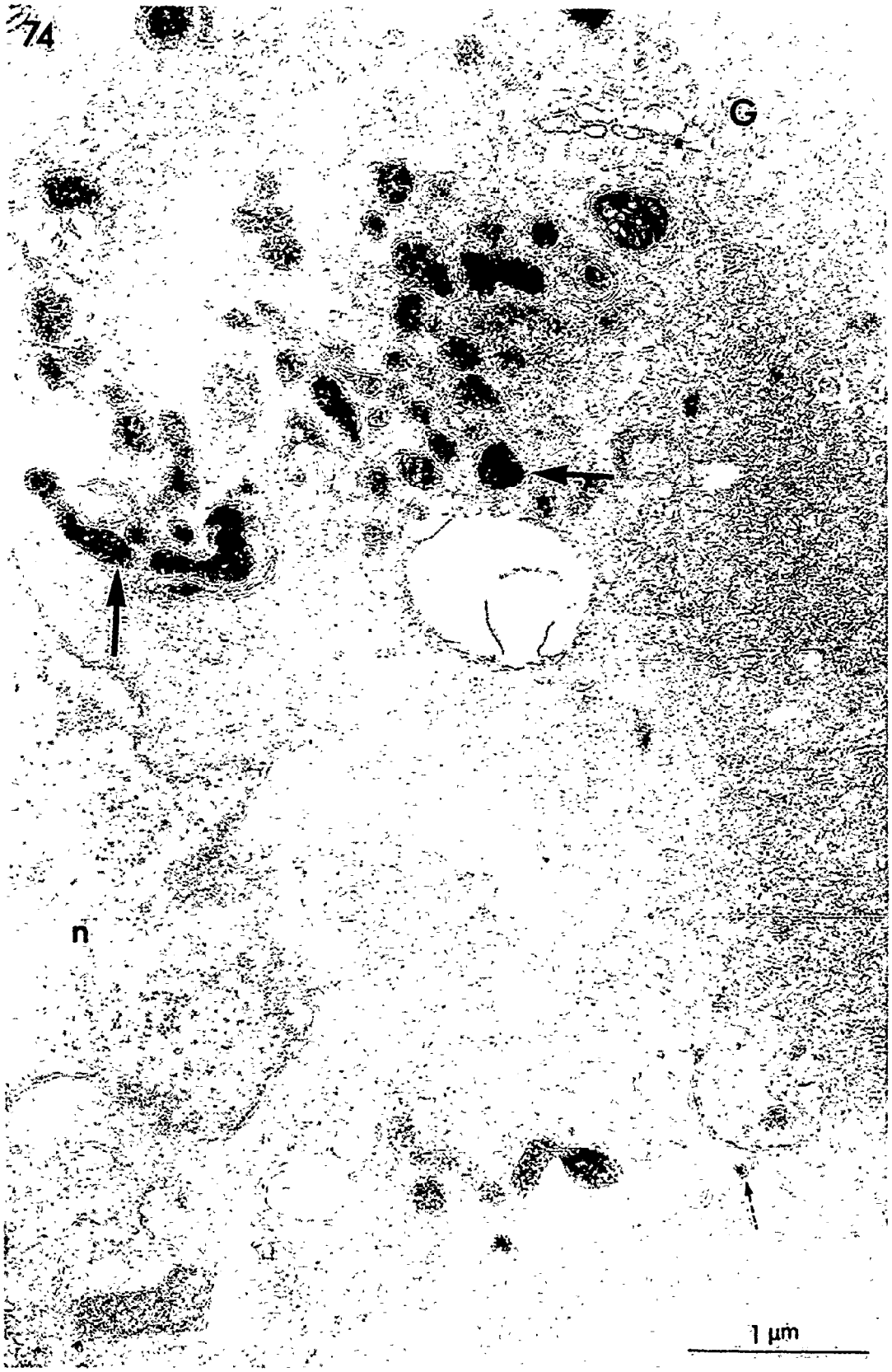
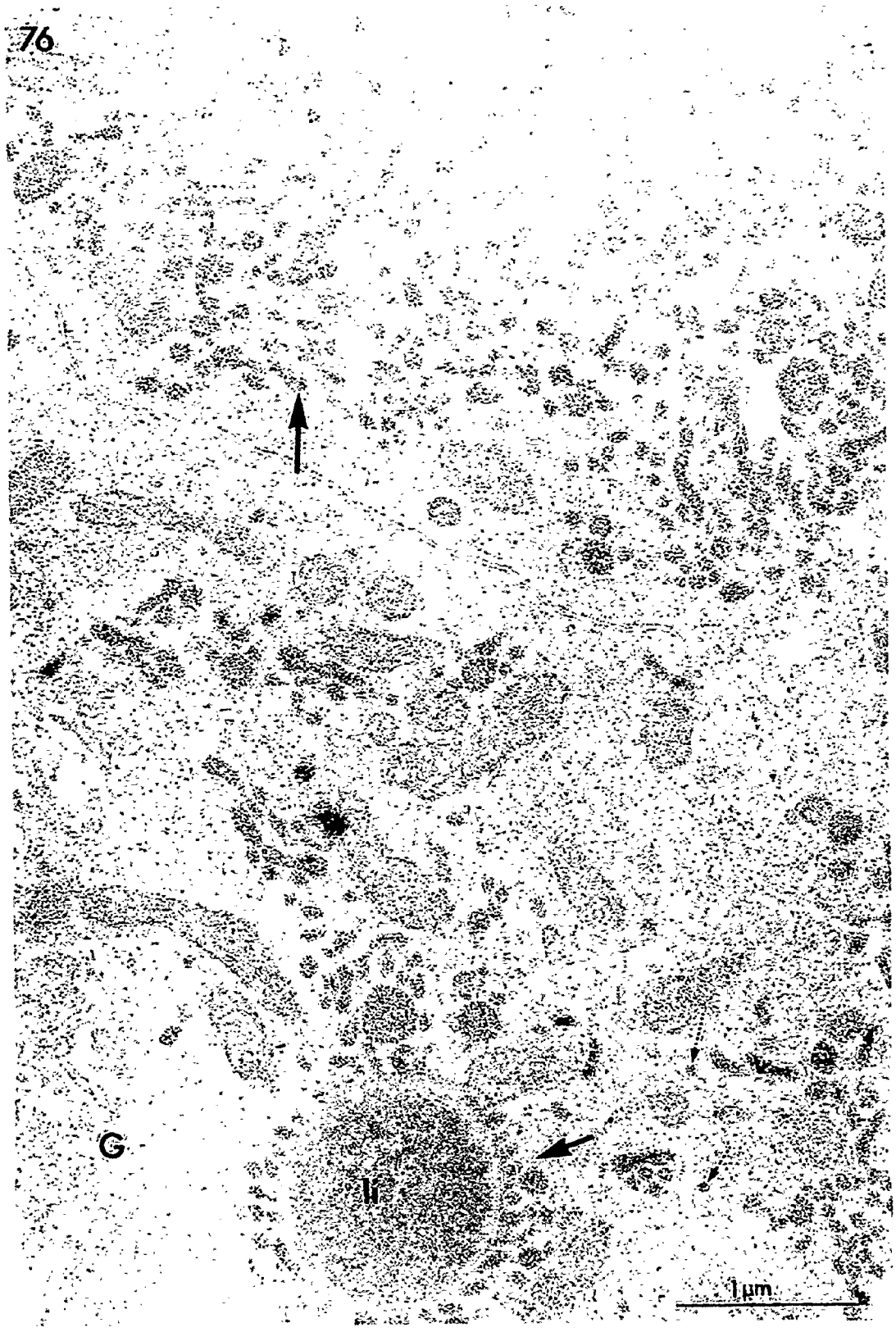

74
G
n
1 µm

Fig. 75. Cabbage looper. Low magnification of positive reaction for catalase as exhibited by elongate and spherical profiles of microbodies (arrows) when incubated in alkaline DAB medium containing hydrogen peroxide. Note the immense number of microbodies, particularly in the vicinity of lipid spheres. 8670X. Age, EC + 3 days. Lipid sphere, li; mitochondrion, m; nucleus, n.


75
li
li
3 µm

Fig. 76. Cabbage looper. Higher magnification of catalase reaction within microbodies (arrows). 31,750X. Age, EC + 3 days. Golgi complex, G; lipid sphere, li. Broken arrows indicate reaction within smooth endoplasmic reticulum.


76
G
1 µm

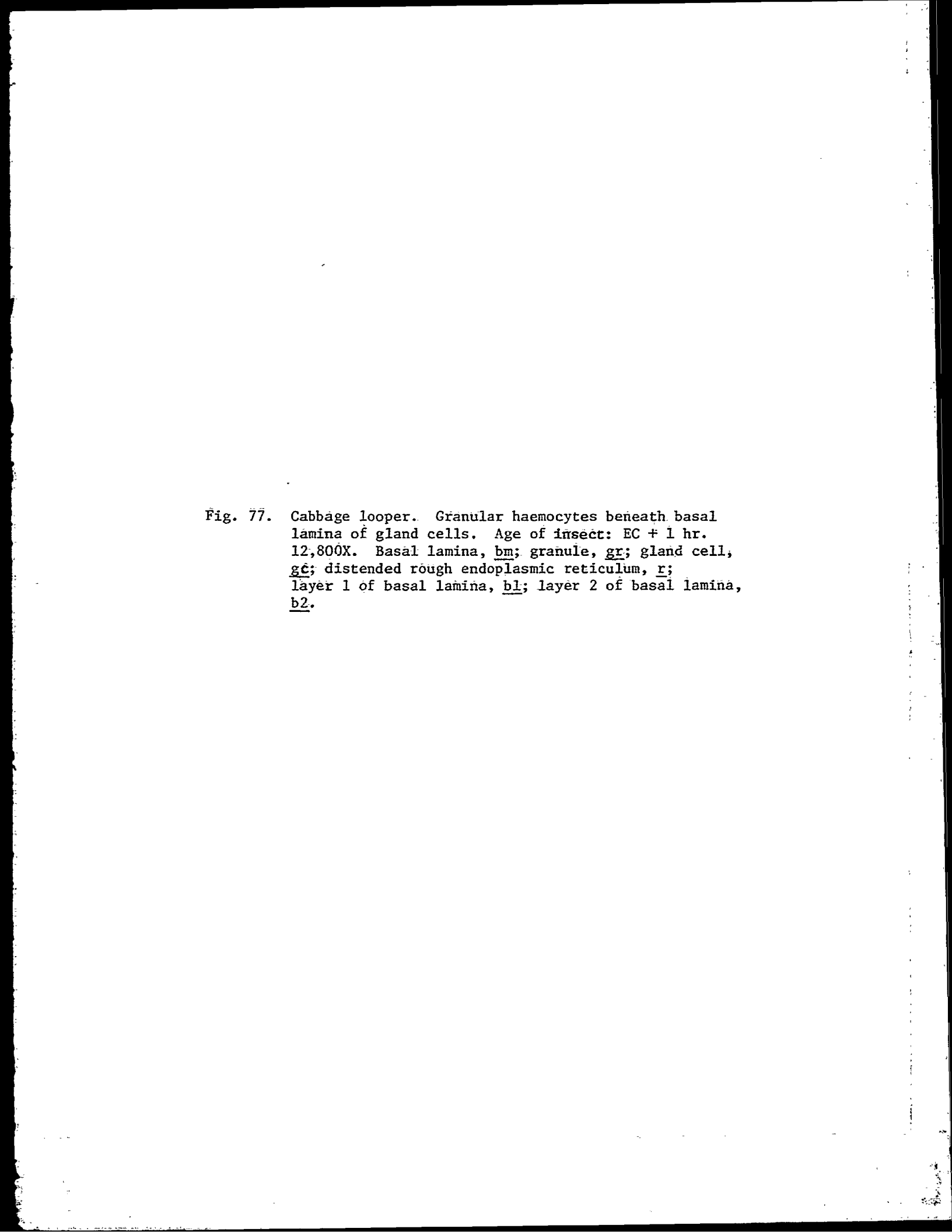


Fig. 77. Cabbage looper. Granular haemocytes beneath basal lamina of gland cells. Age of insect: EC + 1 hr. 12,800X. Basal lamina, bm; granule, gr; gland cell, gc; distended rough endoplasmic reticulum, r; layer 1 of basal lamina, b1; layer 2 of basal lamina, b2.

77

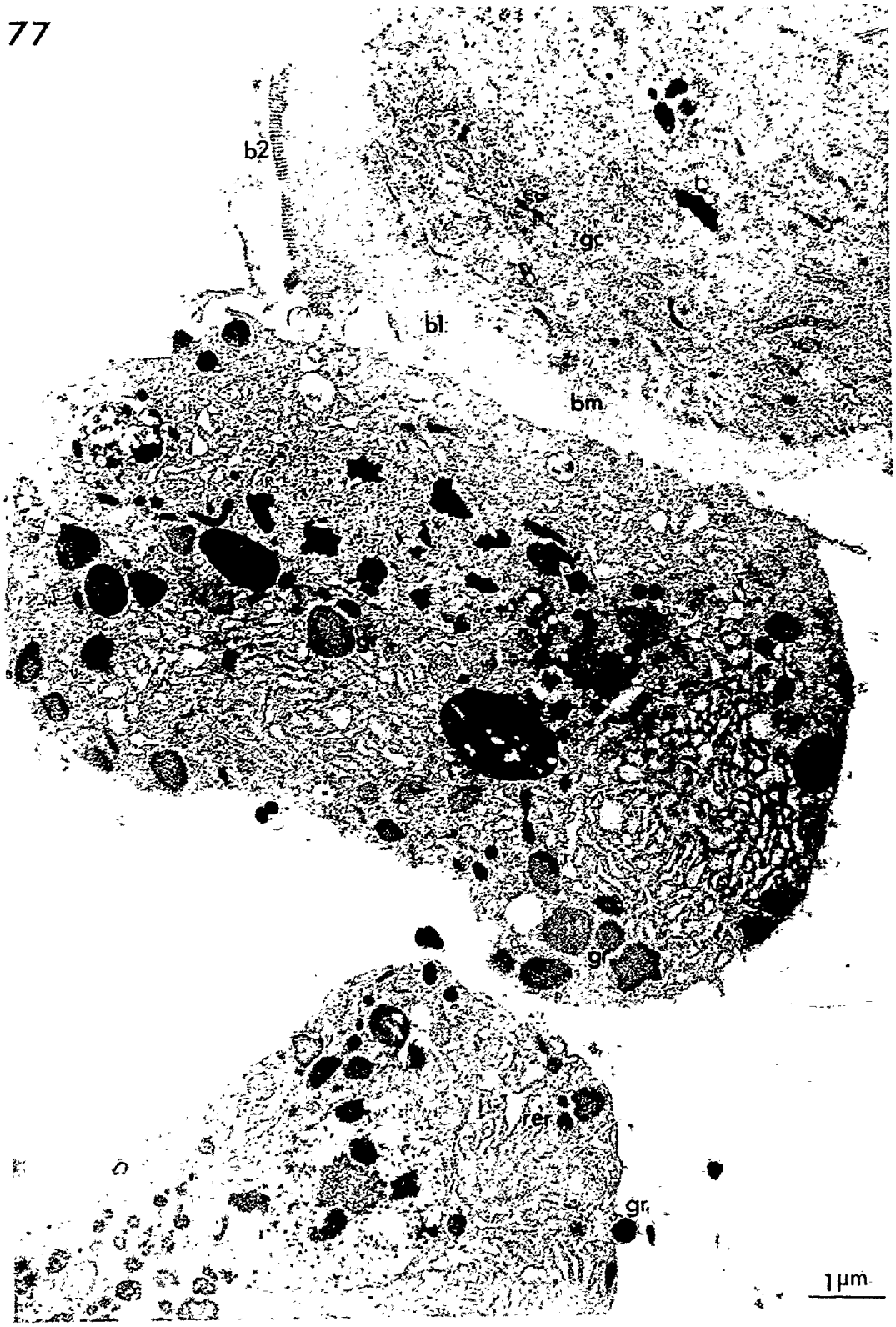


Fig. 78. Cabbage looper. Granules in haemocytes adjacent to basal lamina in which the two layers are not completely organized. Age: EC + 1. hr. 37,500X. Granule, gr; reorganized portion or layer 2, ro; layer 1 of basal lamina, b1; bundles representing layer 2 of basal lamina, b2. Black arrows indicate granules which appear to be emptying their contents into the haemocoel. White arrows indicate tubules of layer 2 traversing layer 1 of basal lamina.

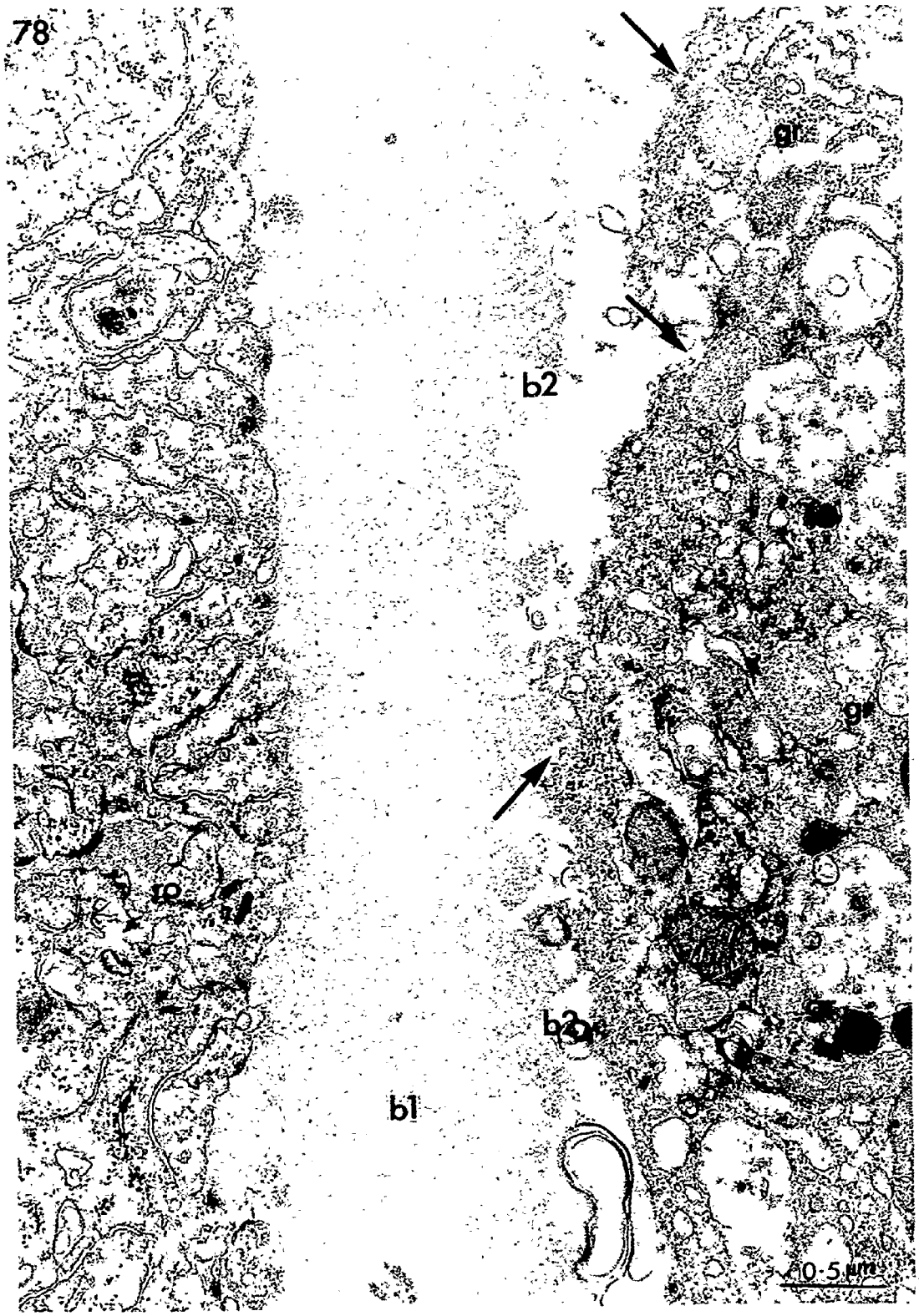
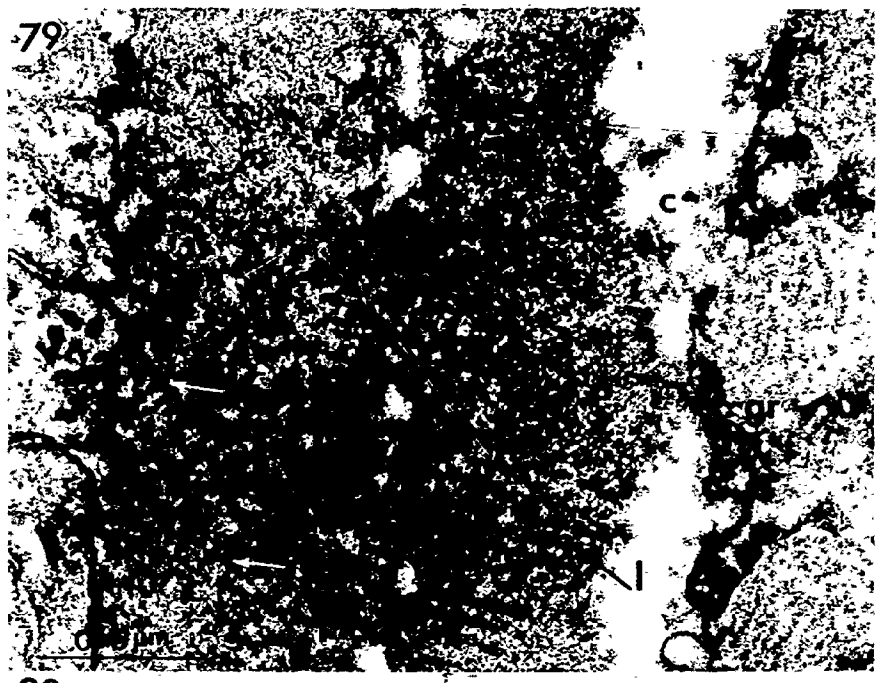

78
gl
b2
gl
b2
b1
0·5 µm

Fig. 79. Cabbage looper. Tubules in granules of haemocytes and in layer 2 of basal lamina. Age: EC +6 hr. 47,750X. Tubules in cross section, tc; granule, gr; tubules in longitudinal section, tl. White arrows indicate tubules of layer 2 crossing layer 1 of basal lamina.

Fig. 80. The proximity of lipid droplets to granules within a granular haemocyte underlying gland cells. Age: 36 hr. pre-eclosion. 34,150X. Granule, gr; Golgi complex, G; lipid, li; distended rough endoplasmic reticulum, rer.


79
c
pr
l

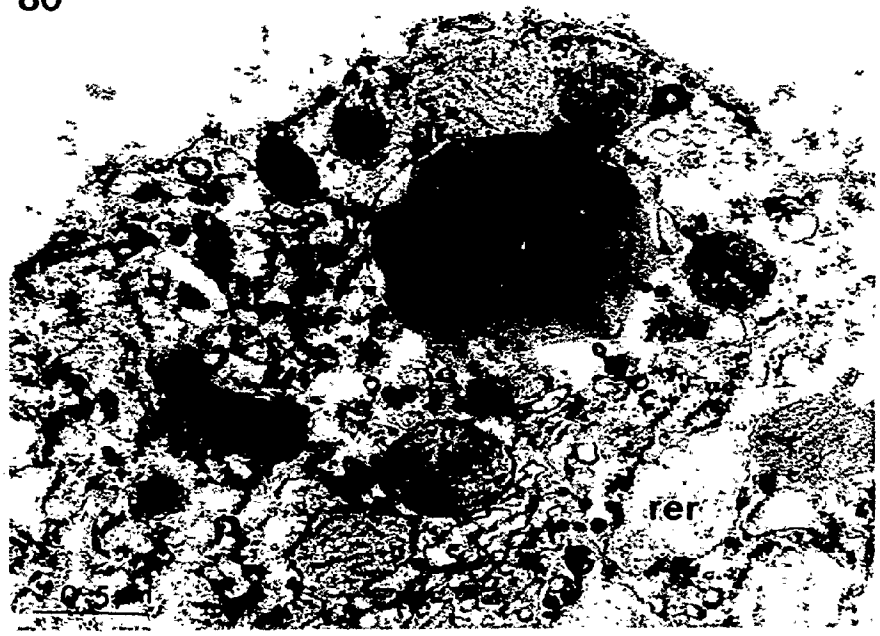

80
rer

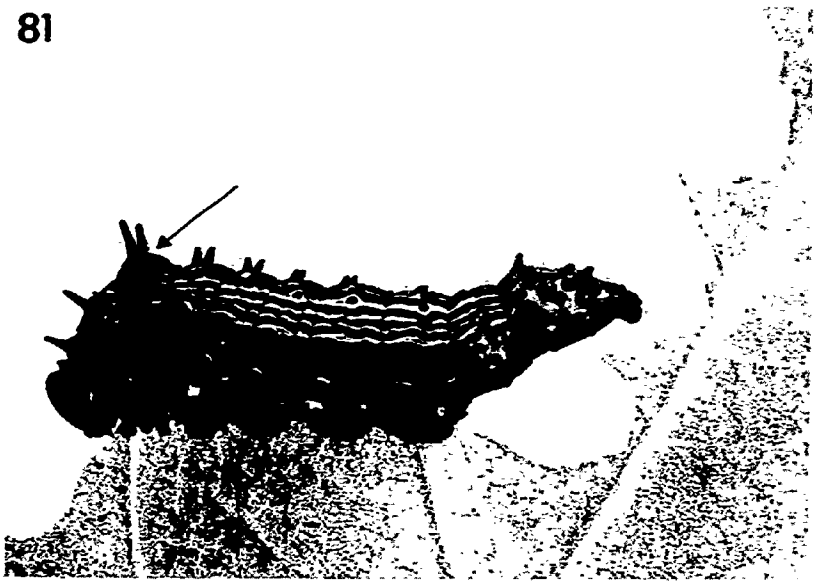
Fig. 81. Red-humped caterpillar in fourth larval instar. Arrow points to 'hump' located dorsally in metathoracic segment.

Fig. 82. Diagrammatic representation of location and size of prothoracic defensive gland in a dissected insect. Anterior gland, AG; gut, Gu; head capsule, HC; interglandular neck, IN; microthorax, MT; prothorax, T ; posterior gland, PG.

The numbers designate the portions of the gland dissected out and prepared for ultrastructeral study.

1. orifice
2. anterior gland
3. anterior lateral longitudinal folds
4. interglandular neck
5. posterior gland - anterior region
6. posterior gland - posterior region

81



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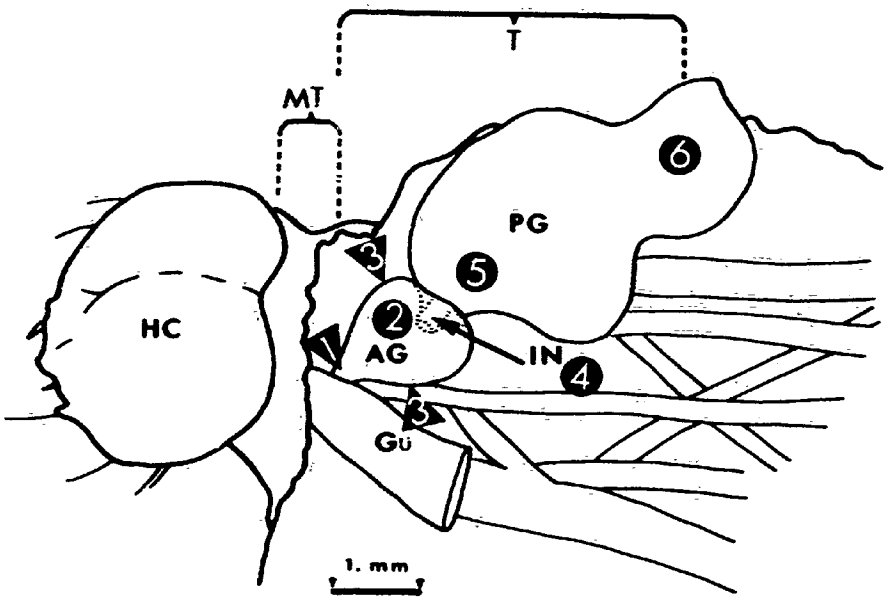
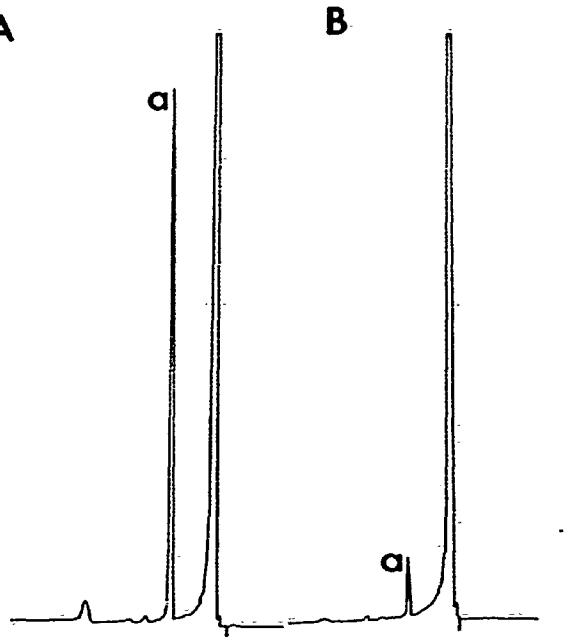


Fig. 83. Red-humped caterpillar defensive secretion. Gas chromatographic trace of compounds present in the anterior gland (A) and posterior gland (B) as detected on a non-polar column. Peak (a) represents the major aliphatic component of this secretion. As can be seem from the peak sizes a greater amount of this compound is present in the anterior gland than is present in the posterior gland.

Fig. 84. Red-humped caterpillar defensive secretion.
(A) Mass spectrum of major peak (a) in the anterior gland.
(B) Mass spectrum of authentic n-decyl acetate.

Peak at m/e 61 is designated because it is a diagnostic peak of acetates representing protonated acetic acid (McLafferty, 1967). The mass spectra of both compounds are identical.

83



84

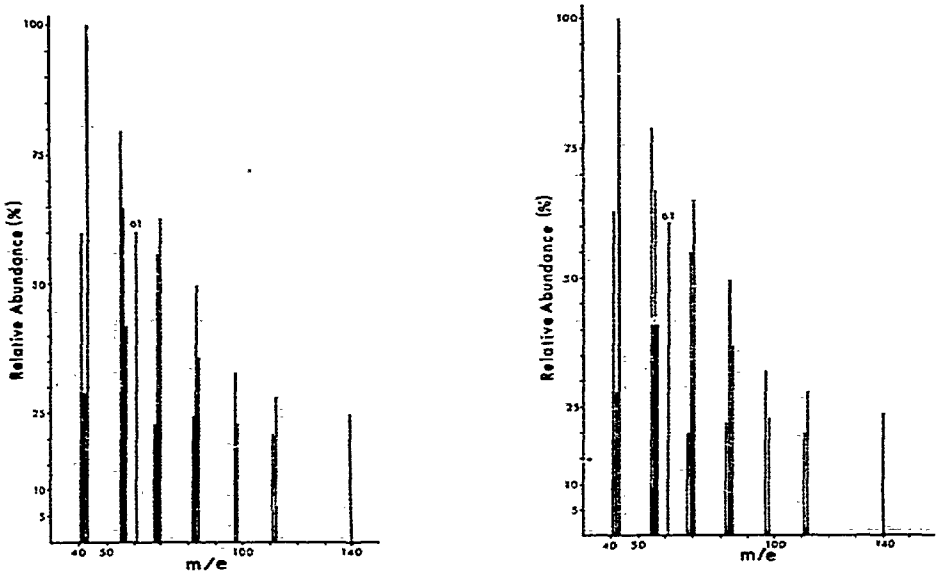


Fig. 85. Red-humped caterpillar. Low magnification of cells near the orifice. 7900X. Basal lamina, bm, dense epicuticle, de; epicuticular filaments, ef; mitochondria, m; nucleus, n; rough endoplasmic reticulum, rer.

85

Fig. 86. Red-humped caterpillar. A typical gland cell representing either the anterior or posterior gland cells. 5500X. Apical folds, af; basal lamina, bm; cuticle, c; gland lumen, L; mitochondria, m; nucleus, n; trough, to.

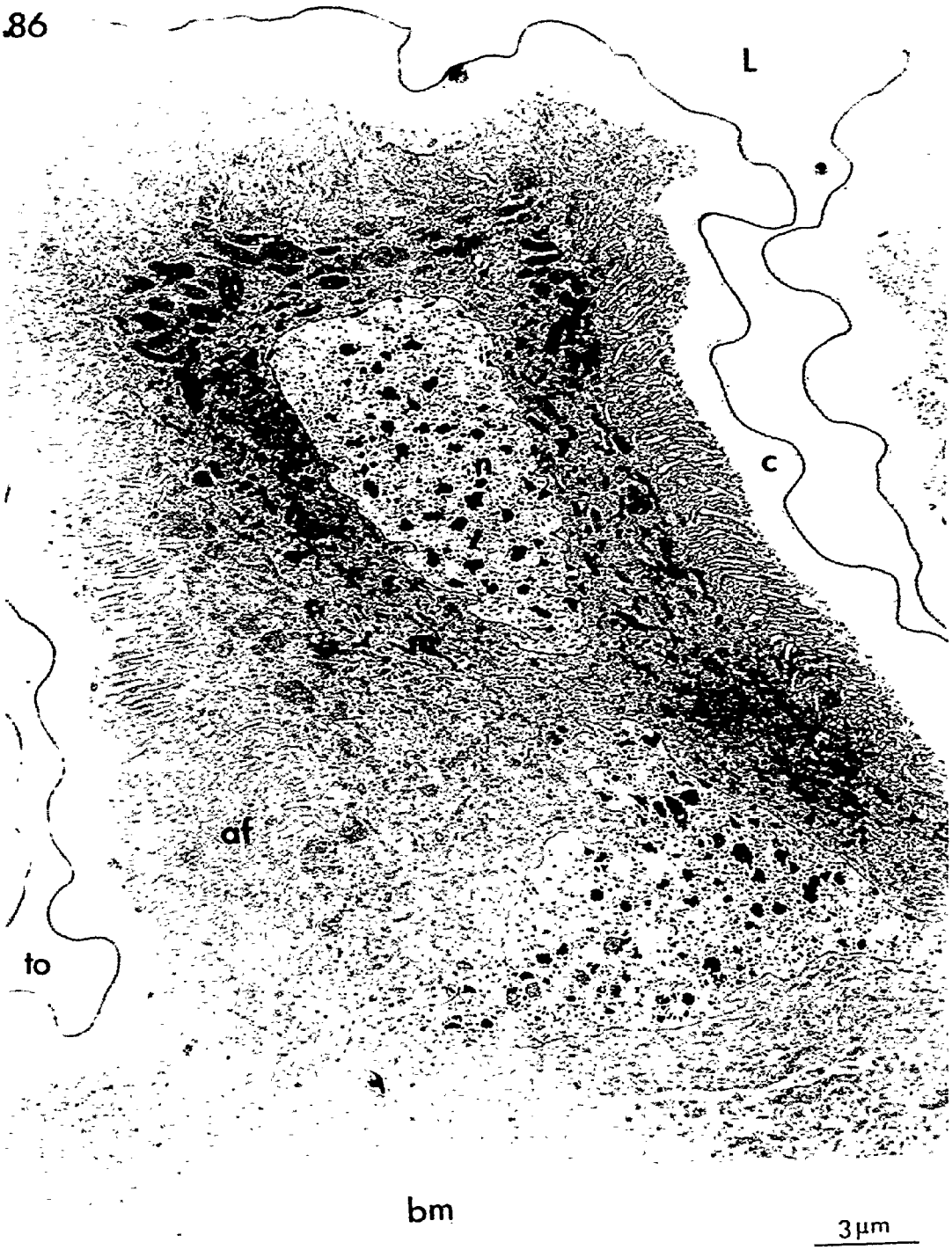

L
c
n
af
to
bm
3 µm

Fig. 87. Red-humped caterpillar. Apical portion of gland cell from anterior gland. Longitudinal section through apical folds. Arrows indicate tubules within the apical folds. (These are seen in cross section in Fig. 88). The smooth endoplasmic reticulum (ser) labelled near the bases of the folds represent areas where these tubules seem to be formed as extensions of the smooth endoplasmic reticulum.

87

Fig. 88. Red-humped caterpillar. Transverse section through apical folds showing the relationship between tubules (tu) and the inverted cylinders of endocuticle. 79,170X. Endocuticle, en; mitochondrion, m.

88

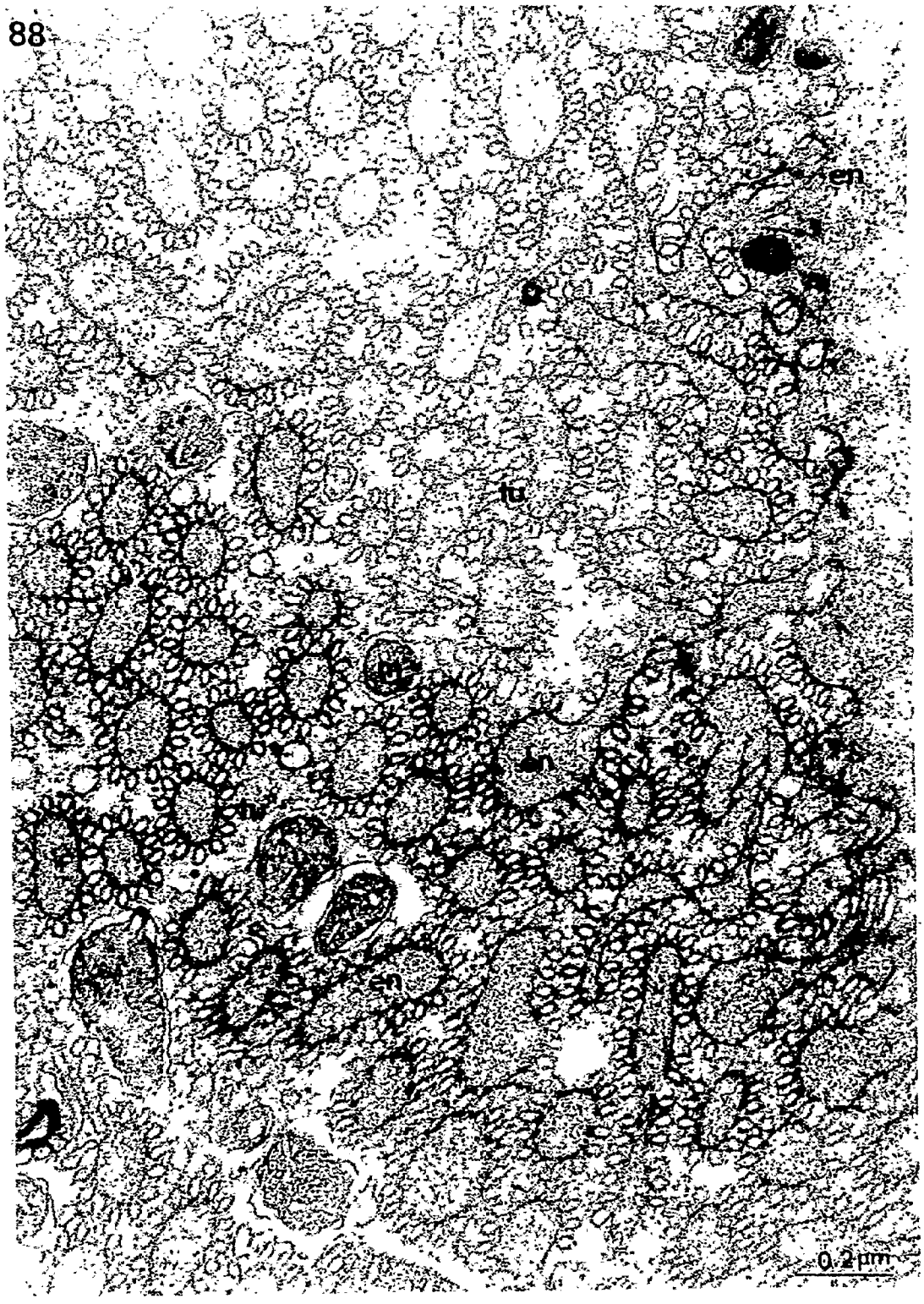


Fig. 89. Red-humped caterpillar. Gland cells from anterior lateral longitudinal folds. Note that these cells contain a deposit of lipid whereas other cells of the anterior and posterior glands do not (Fig. 84). 9600X. Apical folds, af; basal lamina, bm; cuticle, c; lipid, ld; nucleus, n.

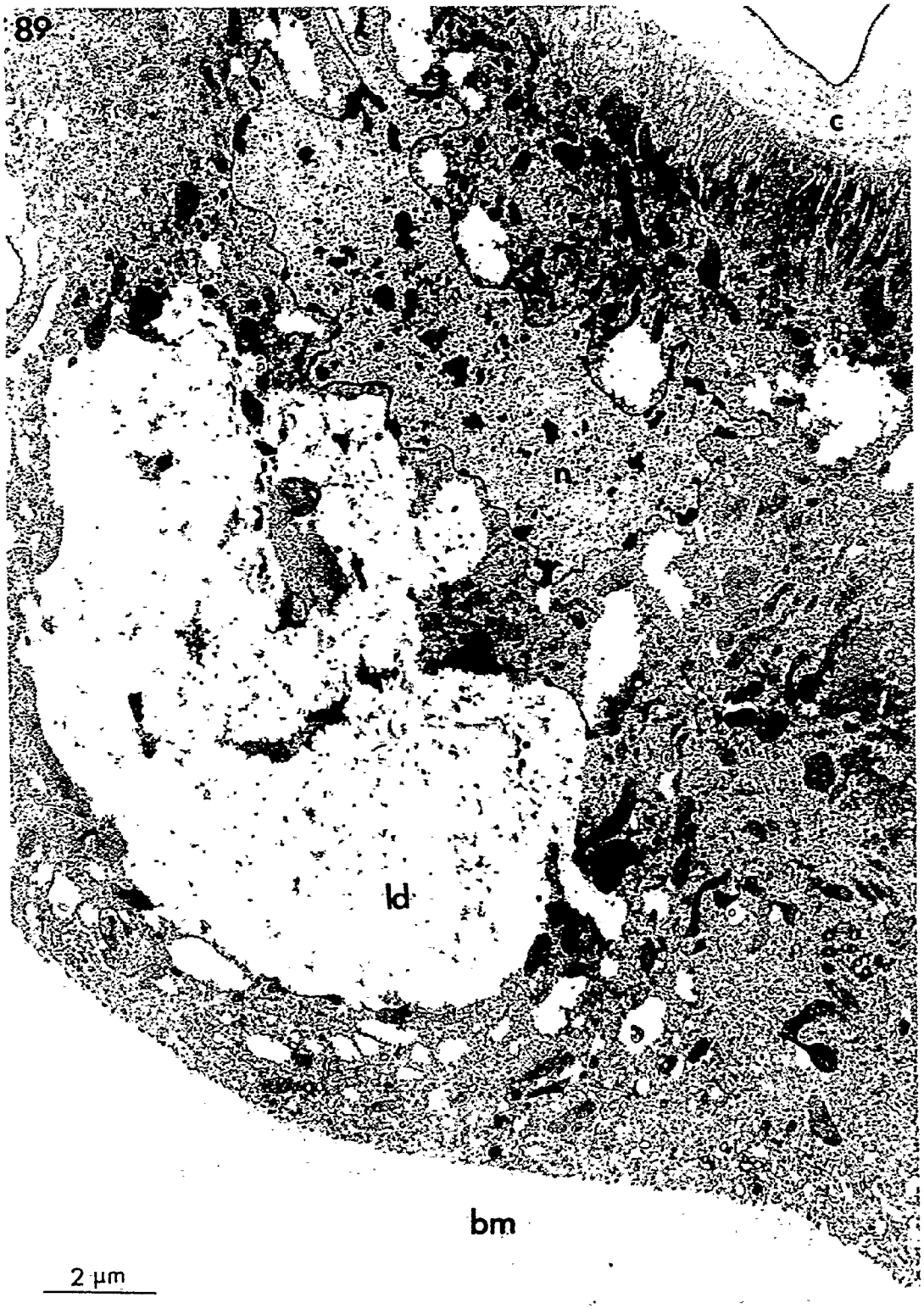

8o
c
n
ld
bm
2 µm

Fig. 90. Red-humped caterpillar. Survey micrograph of cells from interglandular neck. These cells do not have the same folding of the apical membrane as observed in anterior gland cells (Figs. 84, 89). 10,600X. Basal lamina, bm; basal involution, bi; endocuticle, en; lipid deposit, ld; microtubules mt; rough endoplasmic reticulum, rer;


90
en
mt
ld
bi
bm
2 µm

APPENDICES

Appendix 1 - Definition of Terms

Chemical communication: The process whereby organisms release chemical substances into the environment for the purpose of communicating with organisms of the same or different species. (Law and Regnier, 1971).

Semiochemicals: Chemicals released into the environment conveying the message in chemical communication (from Greek "semeion", a mark or signal) (Law and Regnier, 1971).

Allelochemic effect: The class of interations in transpecific communication which affects the recipients' growth, health, behaviour or population biology but excluding food (from Greek "allelo", each other) (Whittaker, 1970; Whittaker and Feeney, 1971).

Allomones: The semiochemicals involved in the allelochemic effects of transpecific communication which evokes a behavioural or developmental reaction adaptively favourable to the transmitter (from Greek, "alloio", different of another sort, "hormon" from "horman", to excite or stimulate) (Brown, 1968; Brown, et al., 1970). e.g. defensive secretions.

Pheromones: Intraspecific semiochemicals released by animal or plant which cause a specific reaction in a receiving individual (from Greek "pherein", to carry, "horman", to excite). Reception may be olfactory or oral (Karlson and Butenandt, 1959; Wilson, 1965).

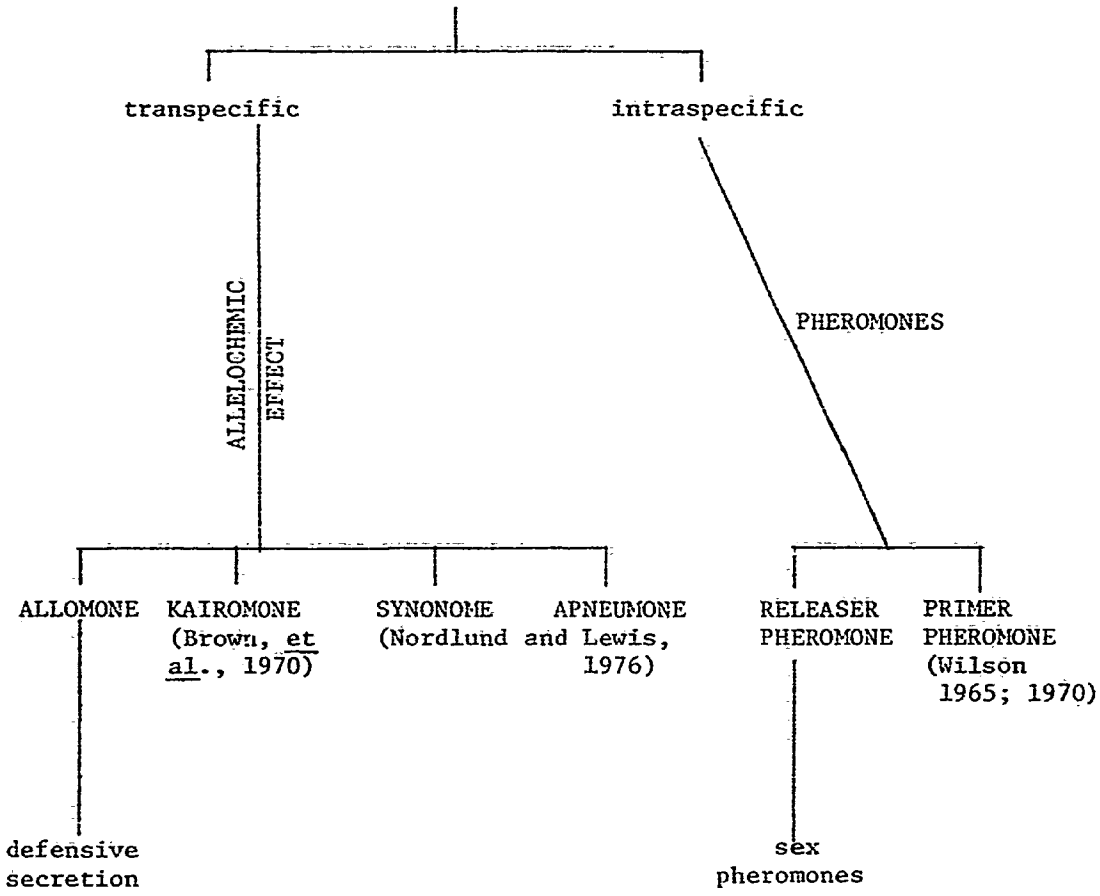
Releaser pheromones: Intraspecific semiochemicals, which cause an immediate behavioural response in the receiving individual (Wilson, 1965; 1970). e.g. sex pheromones.

Appendix 1:

CHEMICAL COMMUNICATION

produces

AIRBORNE SEMIOCHEMICALS



Appendix 2:

Scheme of classification of sex pheromone glands in female adults of the order Lepidoptera. (Modified and extended from that of Urbahn, 1913.)

GROUP I. Ring gland. The entire intersegmental membrane between the eighth and ninth abdominal segments is modified for the production of sex pheromone.

Sub-group (a). The intersegmental membrane folds evenly within the body from dorsal, lateral and ventral surfaces. It is eversible.

Sub-group (b). The intersegmental membrane folds unevenly within the body. The entire gland is eversible. This sub-group includes:

(i) Ring glands with a dorsal sac - the dorsal surface extends within the body cavity further than the other two surfaces.

(ii) Ring glands with a ventral sac - the ventral surface extends within the body cavity further than the other two surfaces.

GROUP II. Dorsal gland. Only the dorsal surface of the intersegmental membrane is modified for the production of sex pheromone.

Sub-group (a). Dorsal fold - when retracted the gland forms a shallow horizontal fold. This sub-group includes:

(i) Dorsal folds which are eversible.

(ii) Dorsal folds which are protrusible.

Sub-group (b). Dorsal sac - when retracted the gland extends deeply within the body cavity. It is eversible. This sub-group includes:

(i) Dorsal sacs which have large longitudinal folds

(ii) Dorsal sacs which do not have longitudinal folds but form a thick compact layer which follows the general outline of the invagination.

GROUP III. Ventral gland. Only the ventral portion of the intersegmental membrane is modified for the production of sex pheromone. It is eversible. In all specimens of this group which have been examined the gland is represented by a ventral sac.

GROUP IV. Ventrolateral glands. The ventrolateral portions of the intersegmental membrane is modified for the production of sex pheromone. The paired glands are sacs and are eversible.

GROUP V. Glandular field. Only a small area within the entire intersegmental membrane is modified for the production of sex pheromone. As a field the gland is protrusible but not eversible.

GROUP VI. Odoriferous internal glands. These are paired, non-eversible glands with a common opening on the papillae anales immediately beneath the openings of the rectum and vagina.

GROUP VII. Glandular tissue located in more than one region of the terminal abdominal segments.

GROUP VIII. Glandular tissue associated with a hairpencil disseminating mechanism.

Appendix 3:

Sex pheromone glands in adult females of lepidopteran families as related to classification scheme outlined on pp. in Appendix 2.

Family	No. of species examined	Type of gland	Reference
Arctiidae	2	VI	Urbahn, 1913.
	1	VII	Urbahn, 1913, MacFarlane and Earle, 1970.
Bombycidae	1	IV	Steinbrecht, 1964a
Gelechiidae	3	II (b)	El-Sawaf, et al.1968; Adeesan, et al., 1969.
Geometriidae	1	IV	Ostaff, et al., 1974.
	1	VI	Werner, 1977.
Lasiocampidae	1	I (b) (i)	Percy and Weatherston, 1971a.
Lymantriidae	7	II (a)(ii)	Urbahn, 1913; Percy et al., 1971.
Noctuidae			
Agrotinae	1	VIII	Urbahn, 1913.
Amphipyrinae	4	I (b)(ii)	Jefferson, et al., 1968; Jefferson and Rubin, 1970.
Heliothinae	2	I (a)(i)	Jefferson et al., 1968.
	1	I (b)(ii)	Jefferson et al., 1968.
Pantheinae	1	I (a)	Urbahn, 1913.
Plusiinae	2	II(a)(i)	Jefferson et al., 1968.
	2	II(b)	Jefferson et al., 1968.
Notodontidae	2	I(b)(i)	Urbahn, 1913.
Pyralidae			
Crambinae	1	II(b)	White and Amborski, 1972.
Phycitinae	1	I(a)	Dickens, 1936.
	3	I(b)(ii)	Dickens, 1936; Weatherston and Percy, 1968; Smithwick, 1970; Fatzinger, 1972,
	2	III	Dickens, 1936.
Saturniidae	3	I(b)(ii)	Urbahn, 1913.

Family	No. of species examined	Type of gland	Reference
Tortricidae			
Olethreutinae	4	II(b)(ii)	George, 1965; Barnes et al., 1966; Roelofs and Feng, 1968.
Tortricinae	6	II(b)(i)	Roelofs and Feng, 1968; Percy and Weatherston, 1971a.
Yponomeutidae	1	I(a)	Thibout, 1972.
	1	VII	Chow et al., 1976.

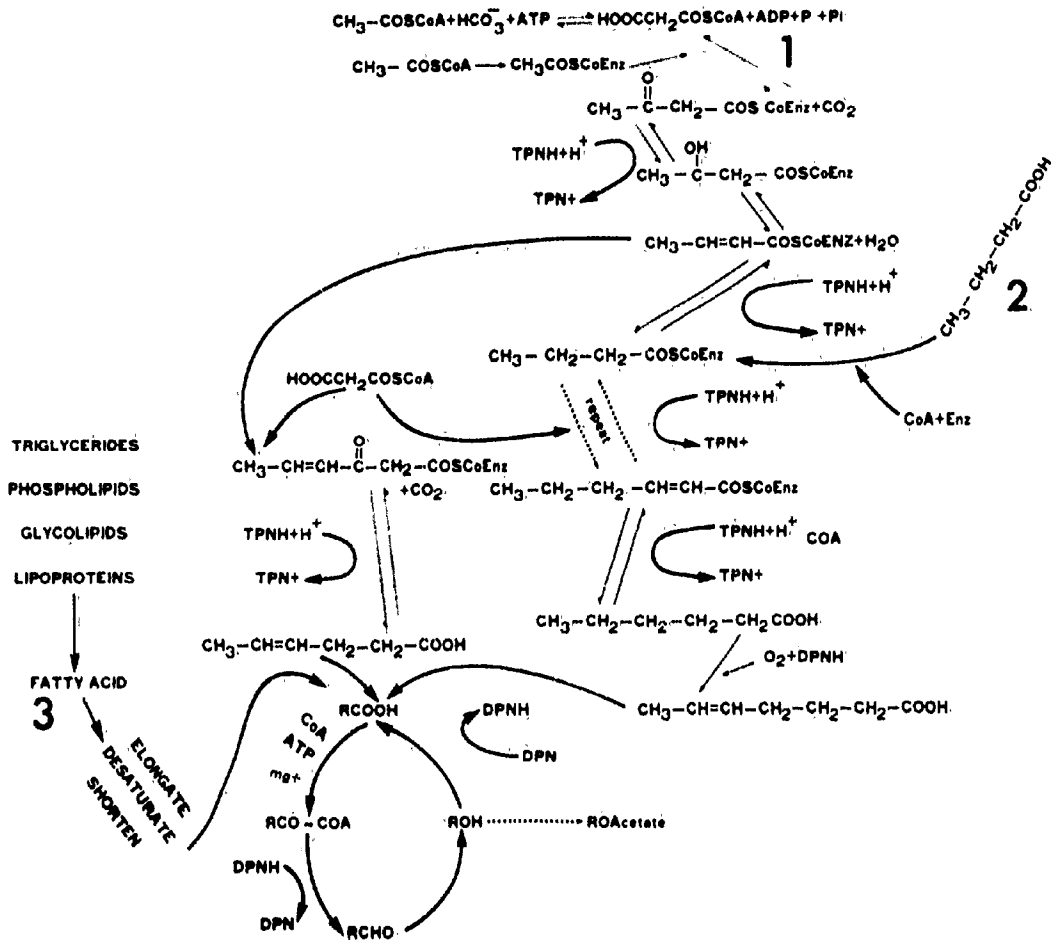
Appendix 4:

Compilation of data related to sex pheromone production by female spruce budworm, cabbage looper and white-marked tussock moth.

	Spruce budworm	Cabbage looper	White-marked tussock moth
Type of gland (from Appendix 3)	II (b)(i)	II (b)(ii)	II (a)(ii)
Calling behaviour	when gland is everted, it remains everted.	when gland is everted, it remains everted.	rhythmic protraction and retraction of terminal abdominal segments.
Age of attractiveness to males	1 to 6 days post-eclosion.	newly eclosed to 6 days post-eclosion.	newly eclosed to 3 days post-eclosion.
Age of maximum attractiveness	2 to 4 days post-eclosion.	2 to 4 days post-eclosion.	12 hours to 1 day post-eclosion.
Attractiveness of mated females	Slight.	Slight.	Slight.
Release rate of pheromone from gland	?	10 ng/min	?
Amount of pheromone per gland	?	5 ng/gland at eclosion, to 500 ng/gland from 2 to 4 days, thereafter declines.	?

	Spruce budworm	Cabbage looper	White-marked tussock moth
Chemical structure of pheromone	96% trans-11-tetradecenal + 4% cis-11 tetradecenal	cis-7-dodecenyl acetate	unknown but probably contains cis-6-henelcosen-11-one
References	Percy and Weatherston, 1971a; Sanders, 1969, 1971a, b; Sanders and Luciuk, 1972a, b; Sanders et al., 1972; Sanders and Weatherston, 1976; Weatherston et al., 1971.	Berger, 1966; Gaston et al., 1966; Henneberry et al., 1967; Ignoffo et al., 1963; Jefferson et al., 1966; Jefferson and Rubin, 1973; Kaae et al., 1973; Kishaba et al., 1970; Saario et al., 1970; Shorey, 1964; Shorey and Gaston, 1965; Shorey et al., 1962; Shorey et al., 1967; Shorey et al., 1968; Sower et al., 1971; Sower et al., 1972.	Grant, 1975; Grant and Frech, 1976; Grant and McCarty, 1977; Percy et al., 1971; Smith et al., 1975.

Appendix 5: Pathways and interconversions involved in the biosynthesis of aliphatic acids and esters (deduced primarily from Wakil, 1970 and Tamaki, 1974. The numbers indicate possible routes using various starting compounds as described in text.


CH3–COSCoA+HCO3−+ATP ⇌ HOOCCH2COSCoA+ADP+P +Pi
CH3– COSCoA → CH3COSCoEnz
1
CH3–C(=O)–CH2– COS CoEnz+CO2
TPNH+H+
TPN+
CH3–C(OH)–CH2– COSCoEnz
CH3–CH=CH–COSCoENZ+H2O
CH3–CH2–CH2–COOH
2
TPNH+H+
TPN+
CH3– CH2–CH2–COSCoEnz
CoA+Enz
HOOCCH2COSCoA
TPNH+H+
TPN+
CH3–CH=CH–C(=O)–CH2–COSCoEnz
+CO2
CH3–CH2–CH2–CH=CH–COSCoEnz
TRIGLYCERIDES
PHOSPHOLIPIDS
GLYCOLIPIDS
LIPOPROTEINS
TPNH+H+
TPN+
TPNH+H+
TPN+
COA
CH3–CH=CH–CH2–CH2–COOH
CH3–CH2–CH2–CH2–CH2COOH
O2+DPNH
FATTY ACID
3
ELONGATE
DESATURATE
SHORTEN
RCOOH
DPNH
DPN
CH3–CH=CH–CH2–CH2–CH2–COOH
CoA
ATP
mg+
RCO ~ COA
ROH ····· ROAcetate
DPNH
DPN
RCHO